

Cleavage sites of endoproteases on the a-chain of GPIb

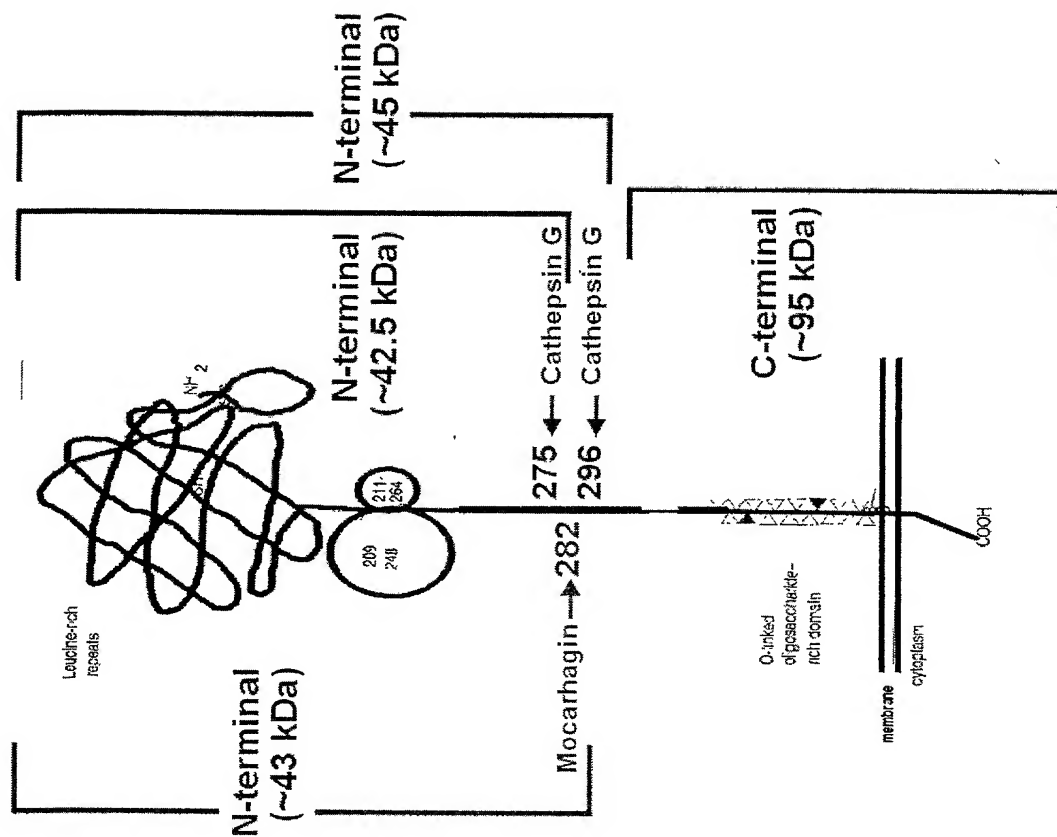
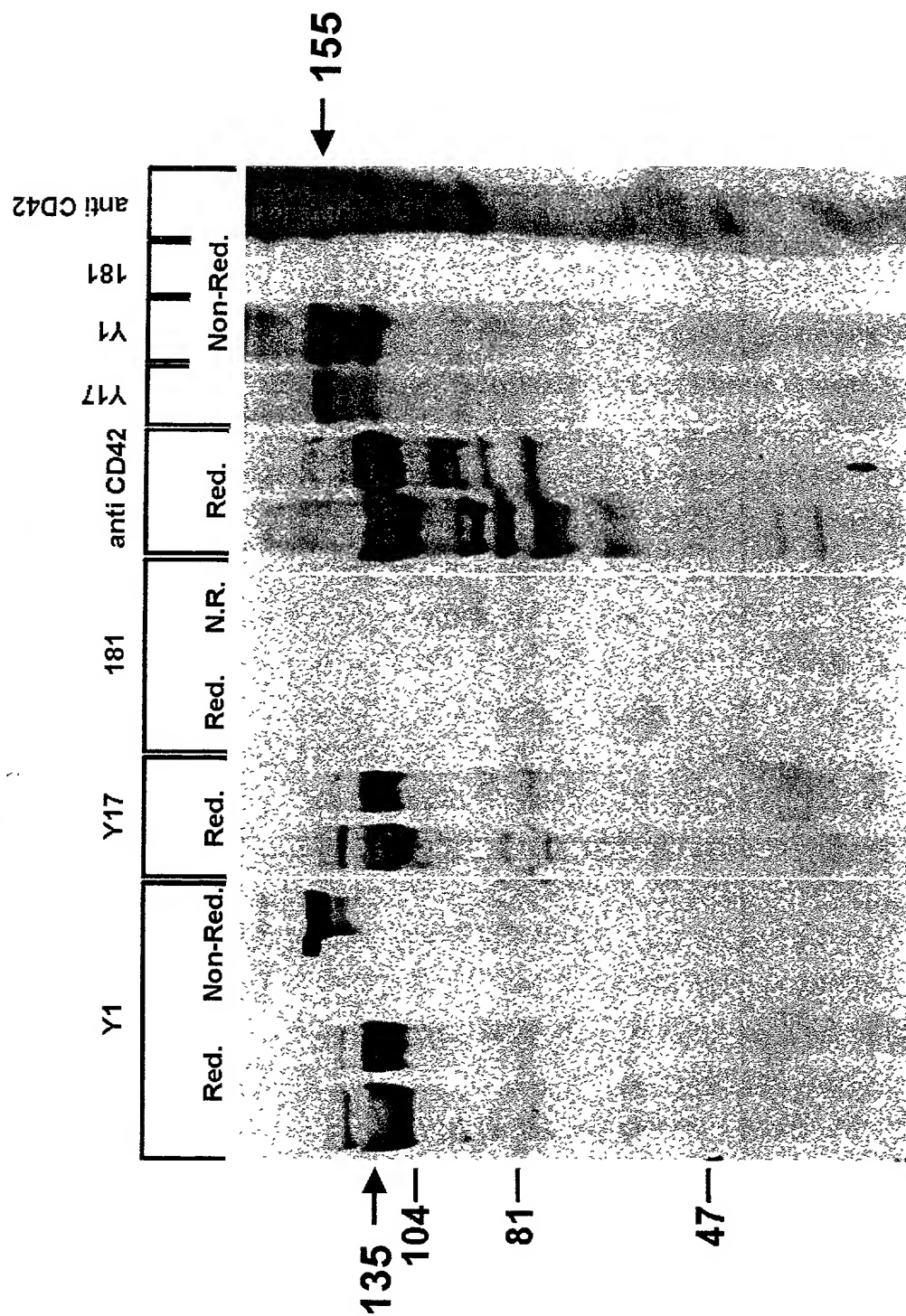


FIG. 1

FIG. 2

Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions



Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

FIG. 3

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

FIG. 4

Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17

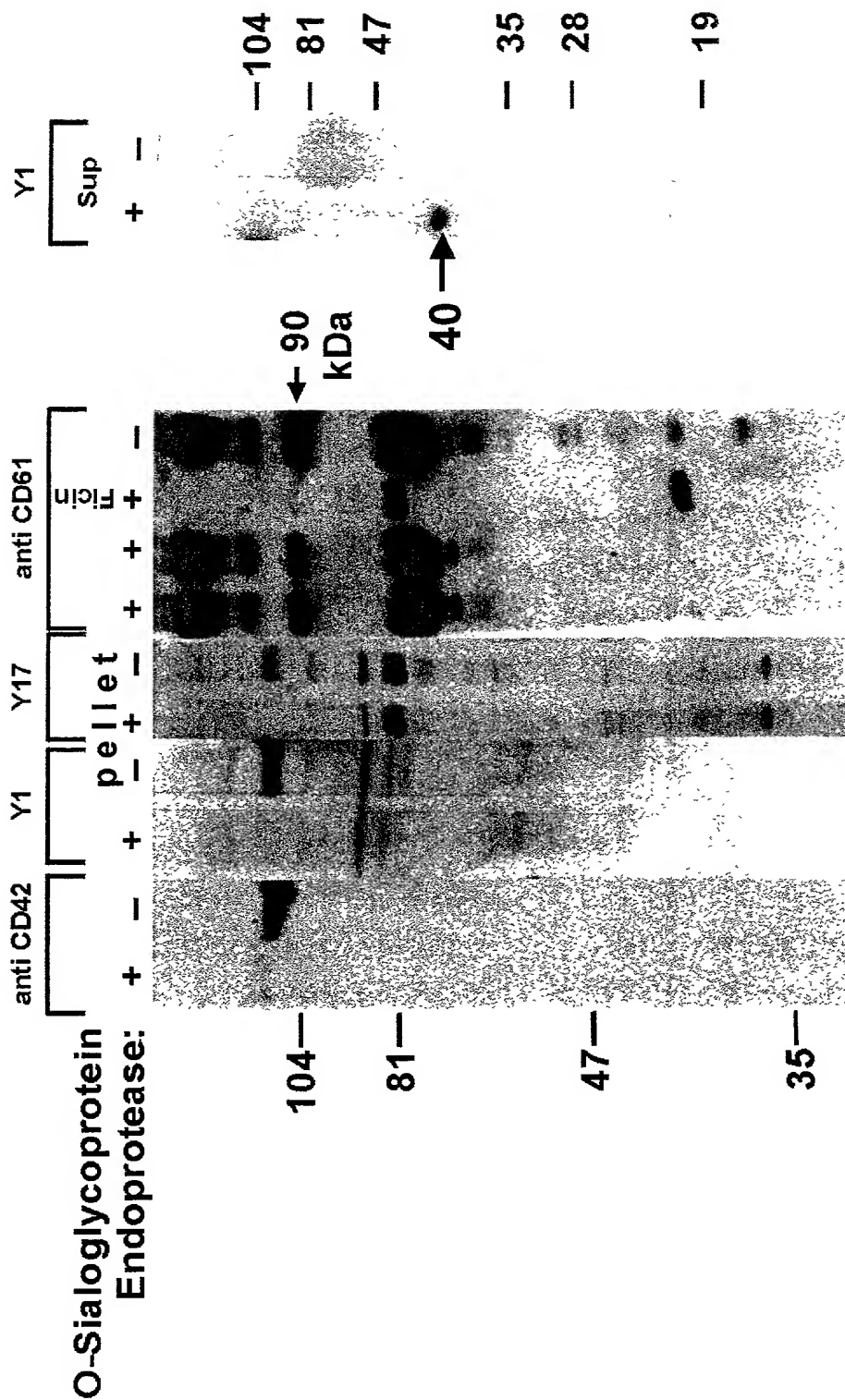
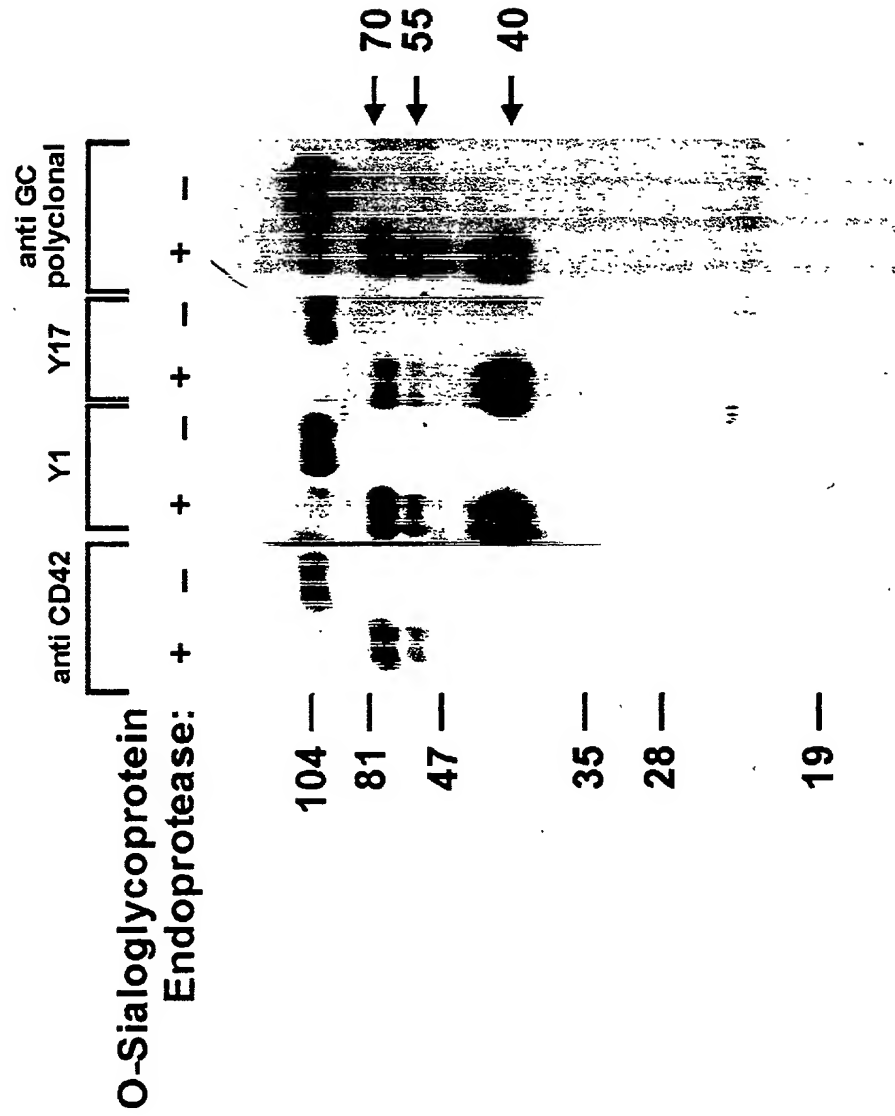
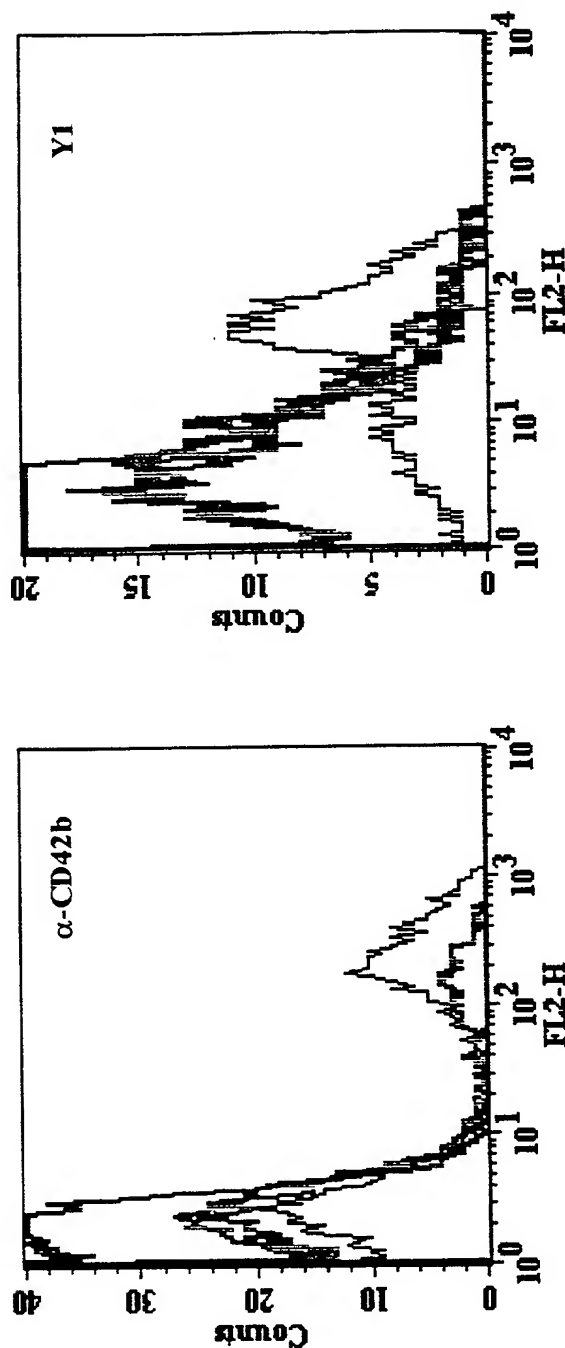


FIG. 5

Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease



Specific GPIIb Proteolysis Abolishes Y1 Binding to Platelets



Key	Name	Parameter	G
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NON-TREATED PLATELETS

— O-SIALOGLYCOPROTEIN ENDO. (10 μg/ml)

— O-SIALOGLYCOPROTEIN ENDO. (50 μg/ml)

— FICIN (18 μg/ml)

mocarhagin:	anti CD42 C-terminal		Y1		anti CD42 N-terminal		CG
	-	+	-	+	-	+	
104—	+	+	+	+	+	+	+
81—	+	+	+	+	+	+	+
47—	+	+	+	+	+	+	+
35—	+	+	+	+	+	+	+
28—	+	+	+	+	+	+	+

FIG. 7

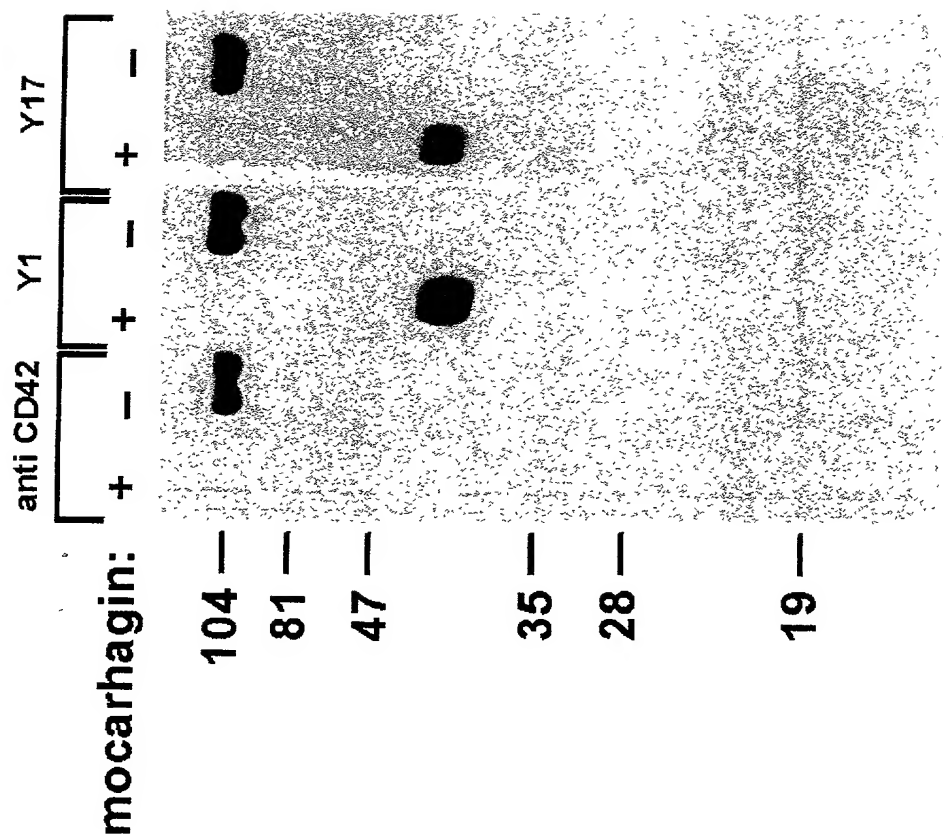
[illegible]

FIG. 9

Binding of Y1 and Y17 to platelets

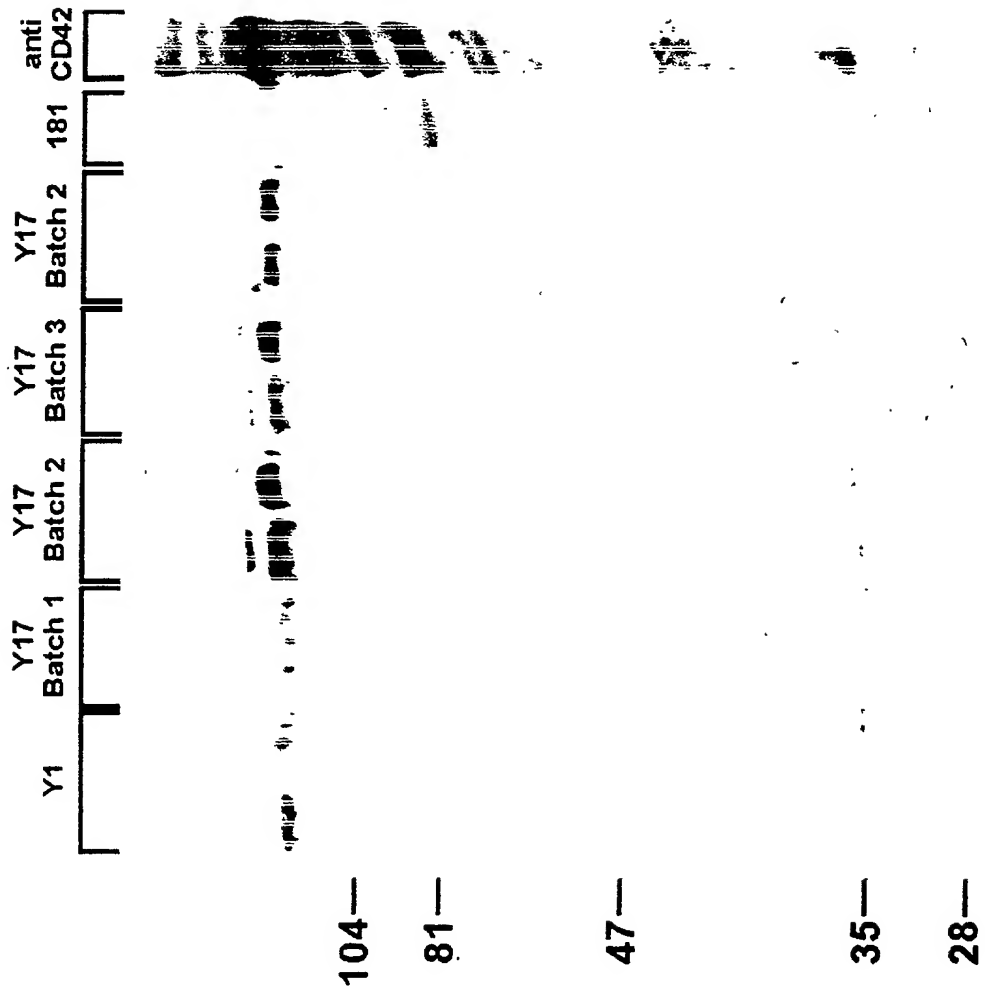


FIG. 10

Y1 and Y17 bind glycoprotein similar after cleavage by Ficin

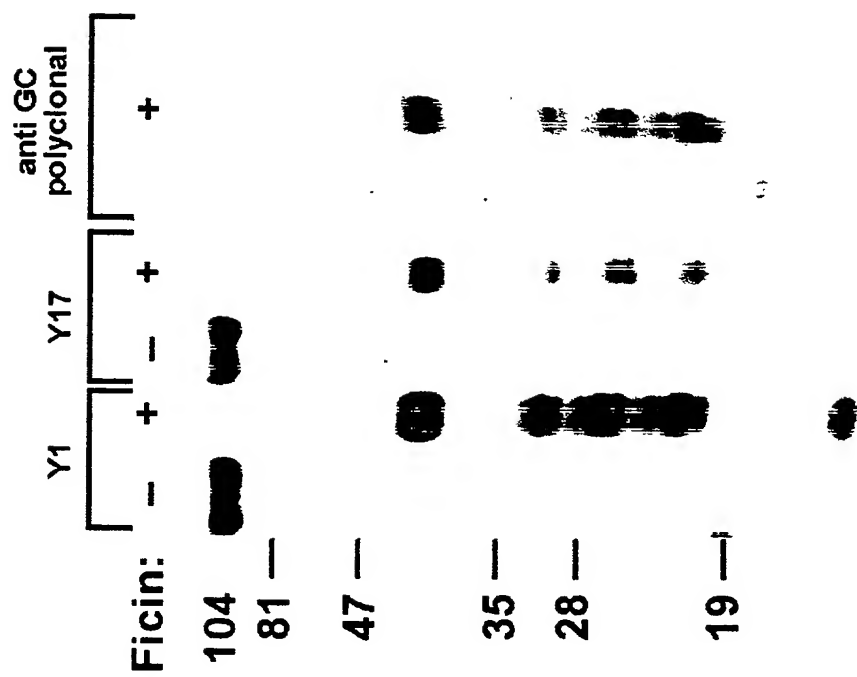


FIG. 11

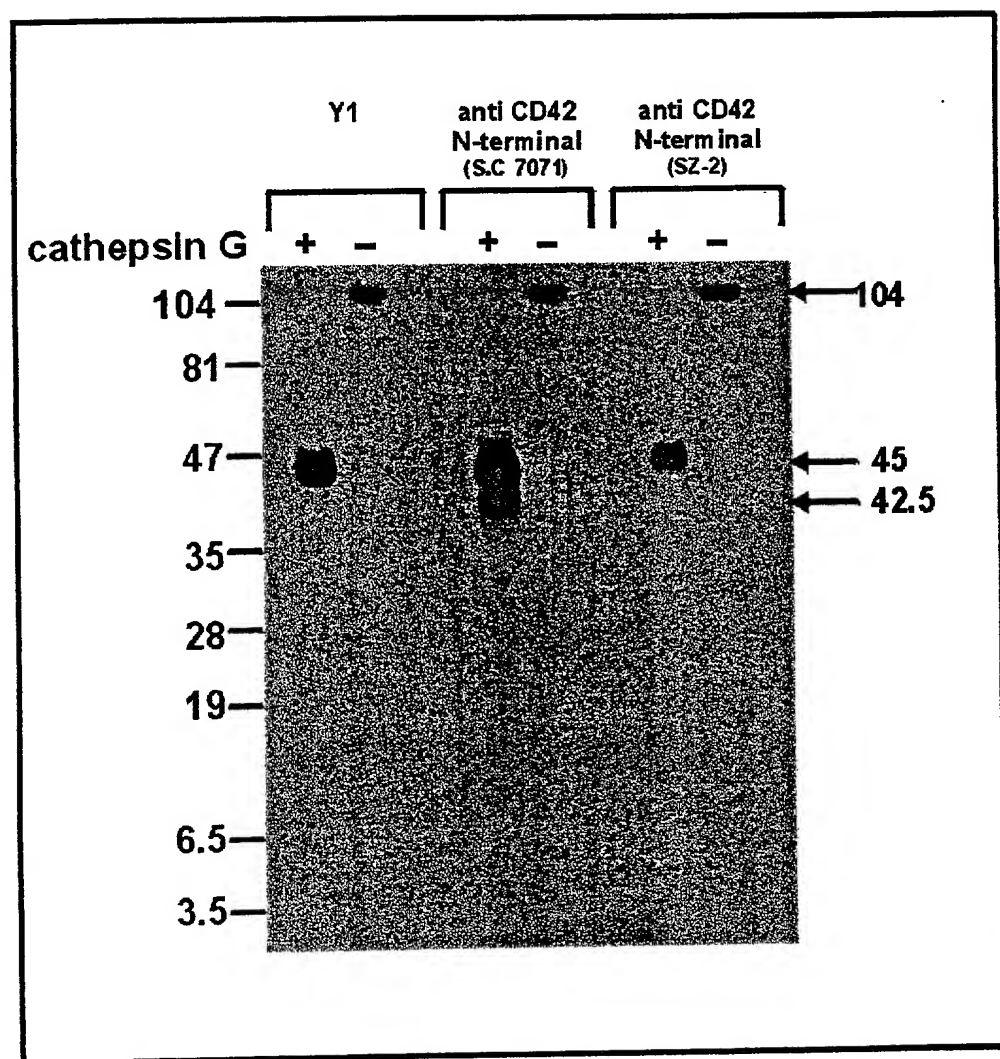


FIG. 12

**Y1 and Y17 reacts with larger cathepsin G cleaved
platelets GPIb fragment**

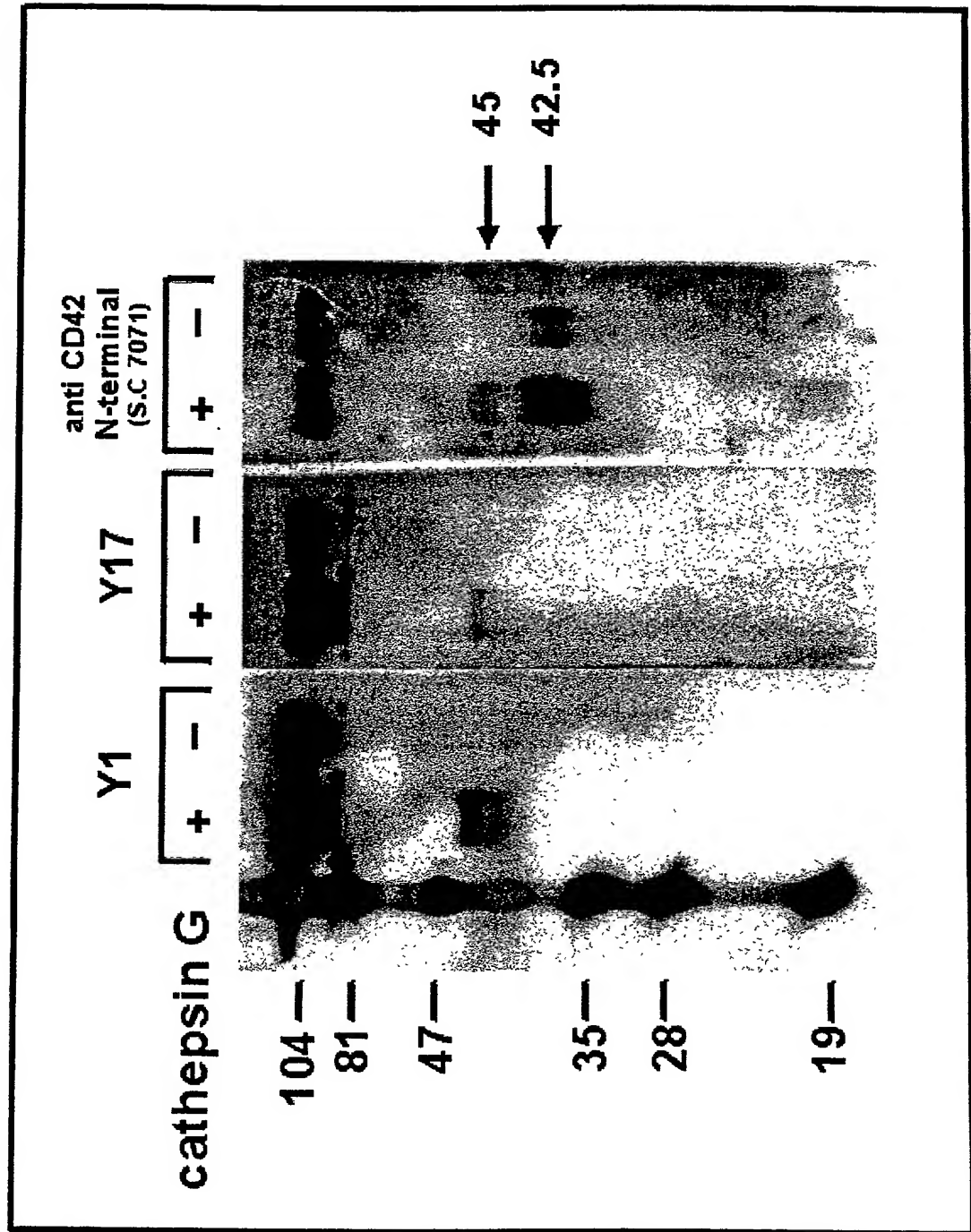
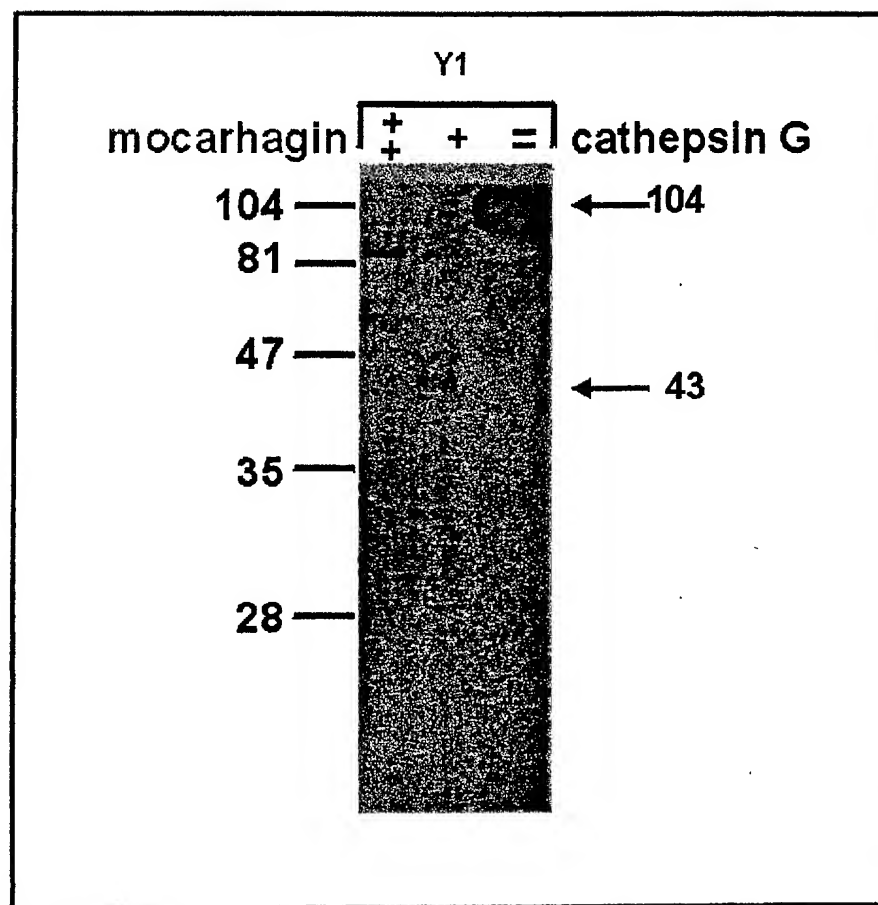


FIG. 13



Cleavage of washed platelets by mocarhagin and cathepsin G

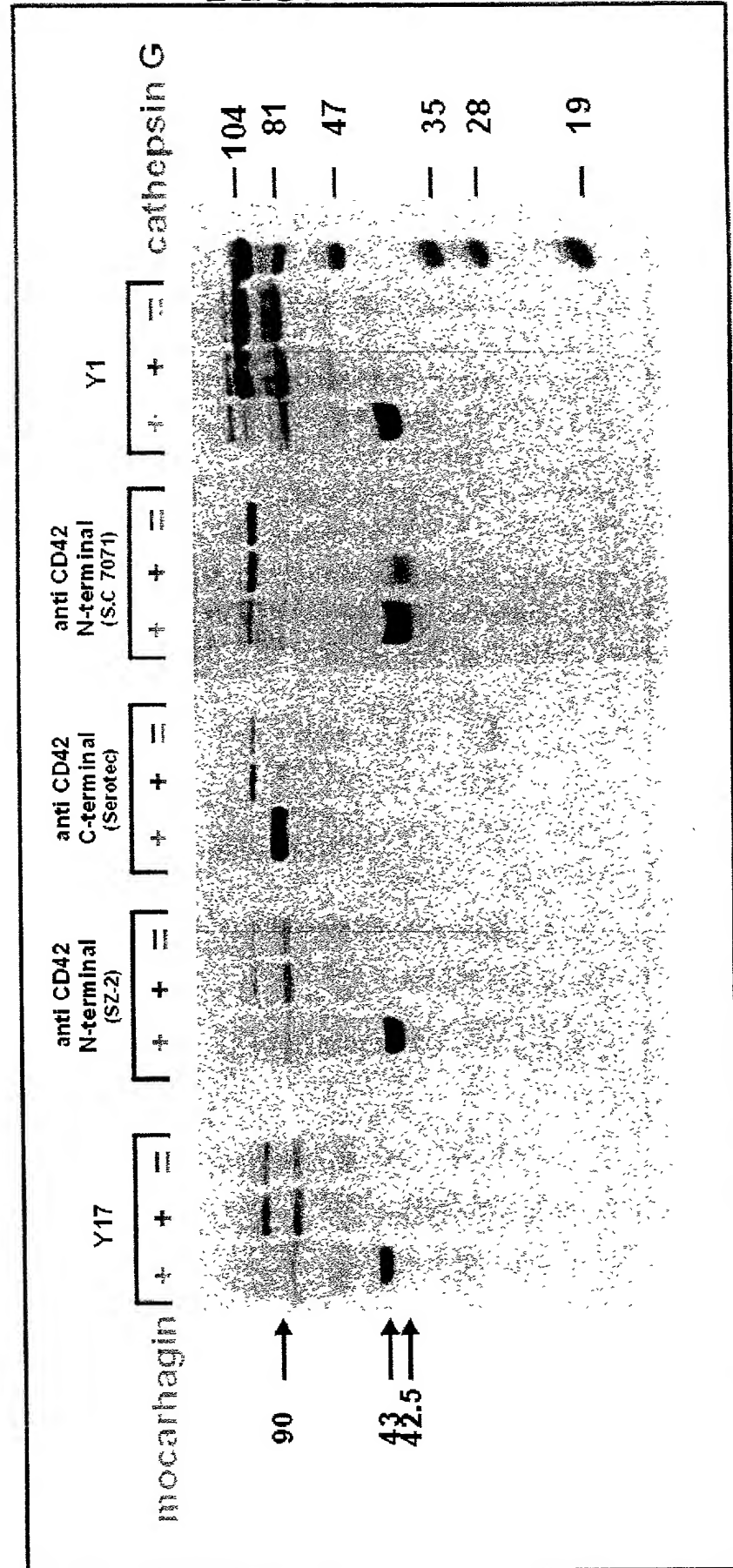


FIG. 15

Influence of Y1-scFv on platelets agglutination in washed platelets

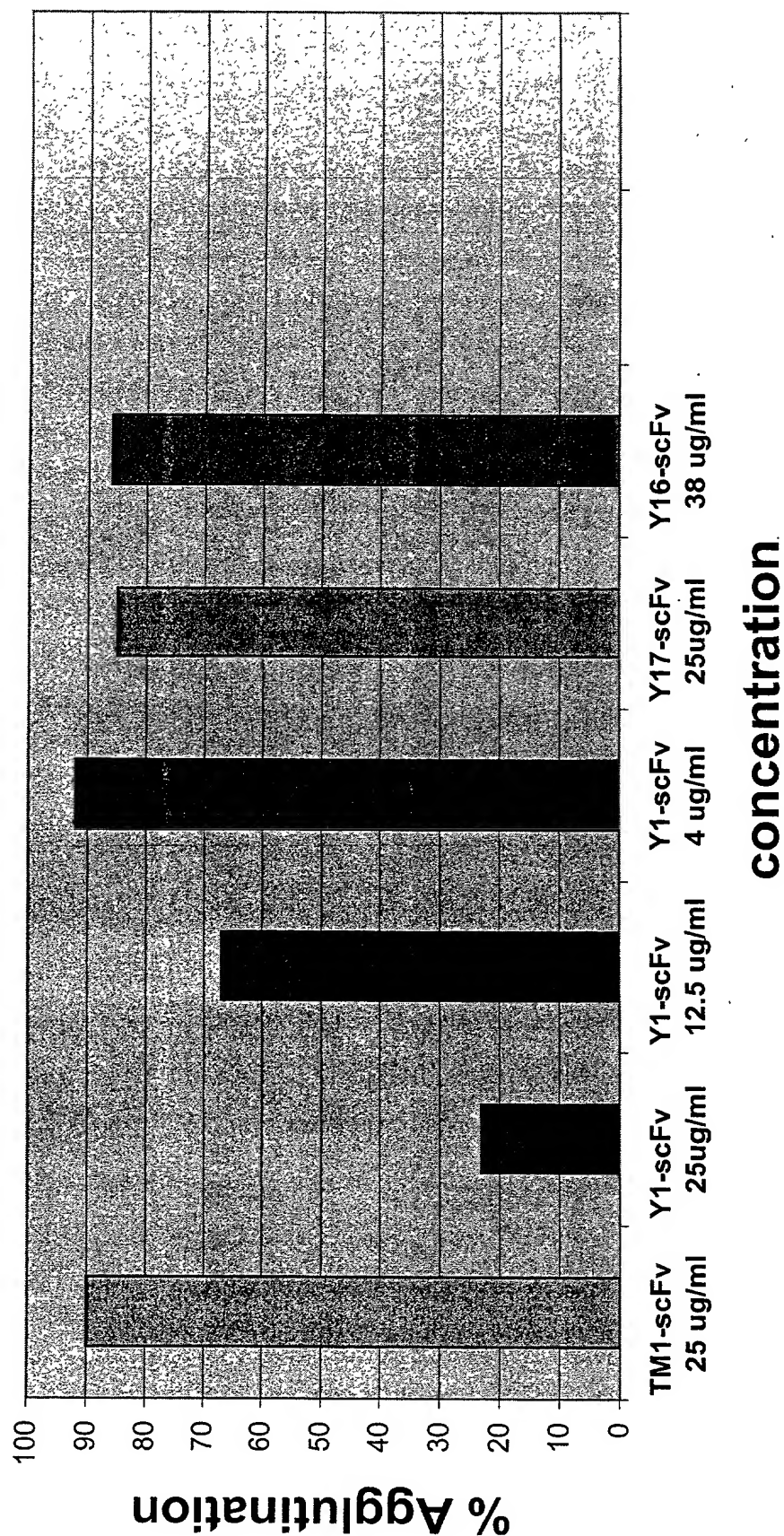


FIG. 16

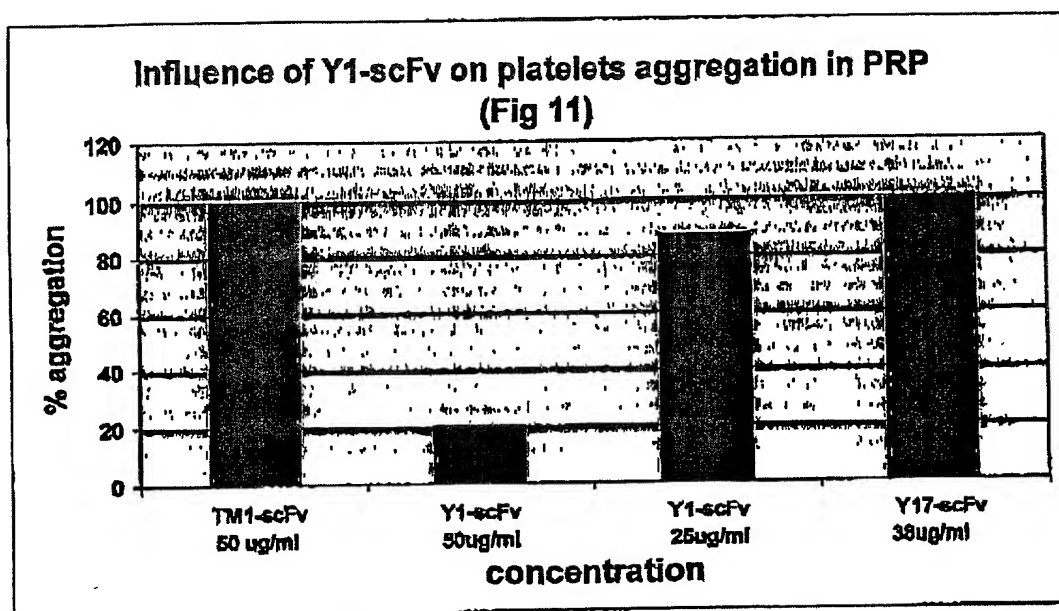


FIG. 17

Induction of platelet agglutination by Y1-IgG in washed platelets

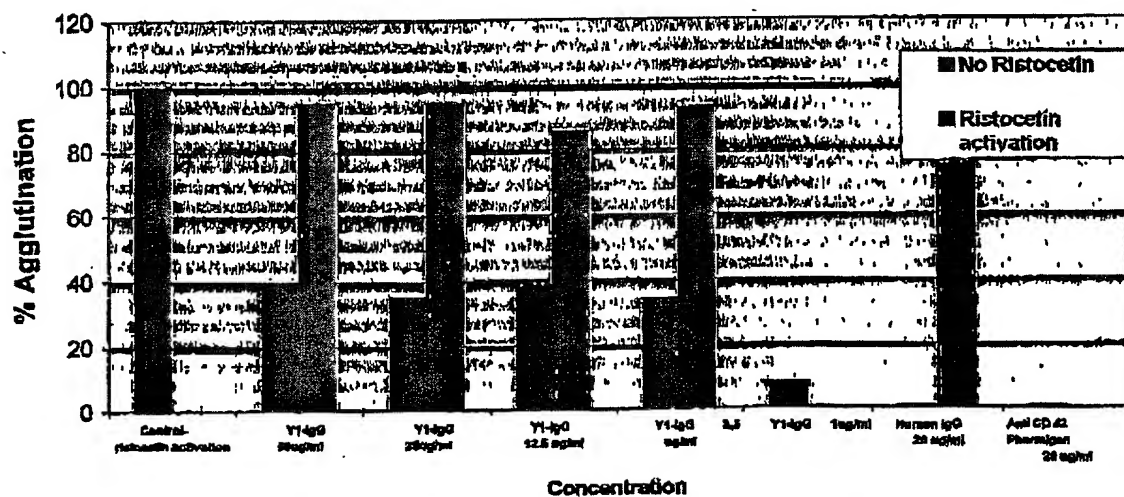


FIG. 18

Induction of platelet aggregation by Y1-IgG in PRP

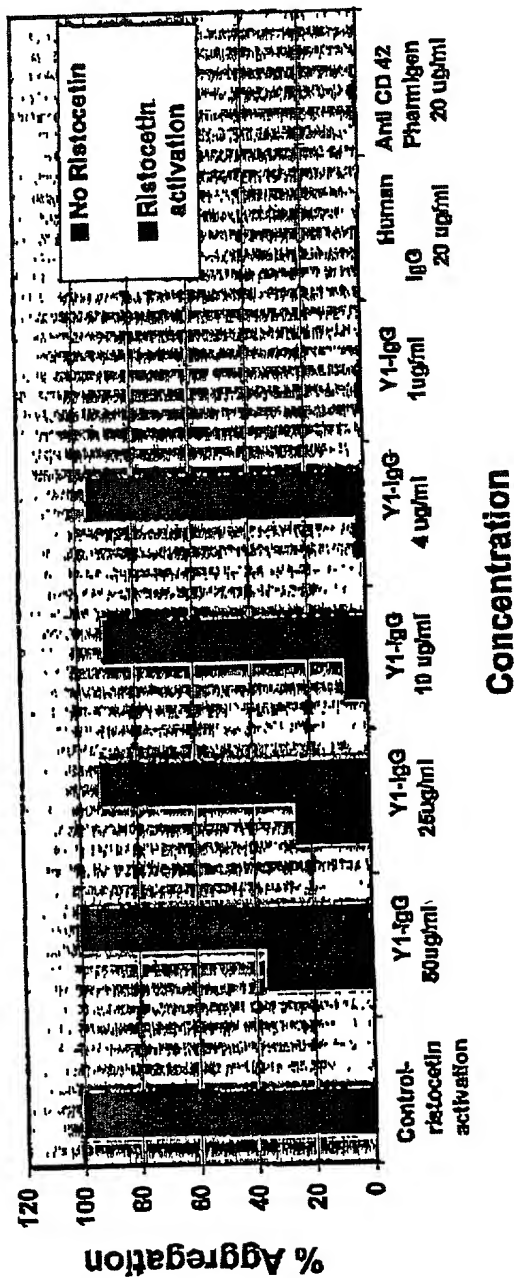
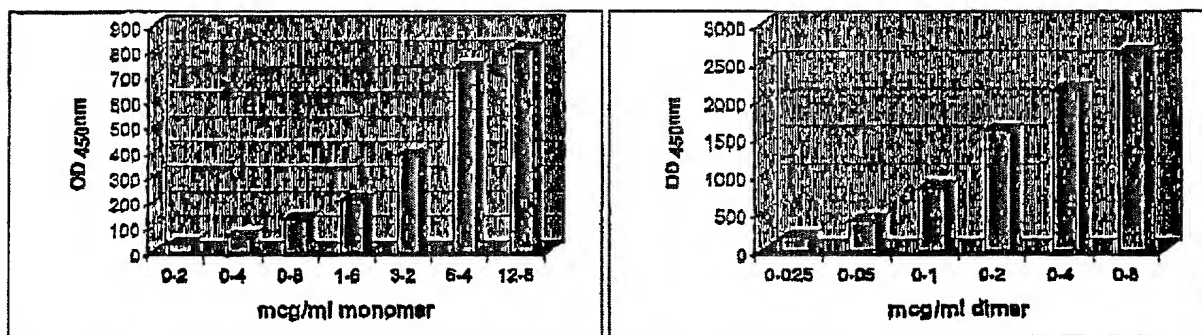


FIG. 19



Specificity of Binding of Y1 and α -CD42 (N1-19) to their Ligands

FIG. 20

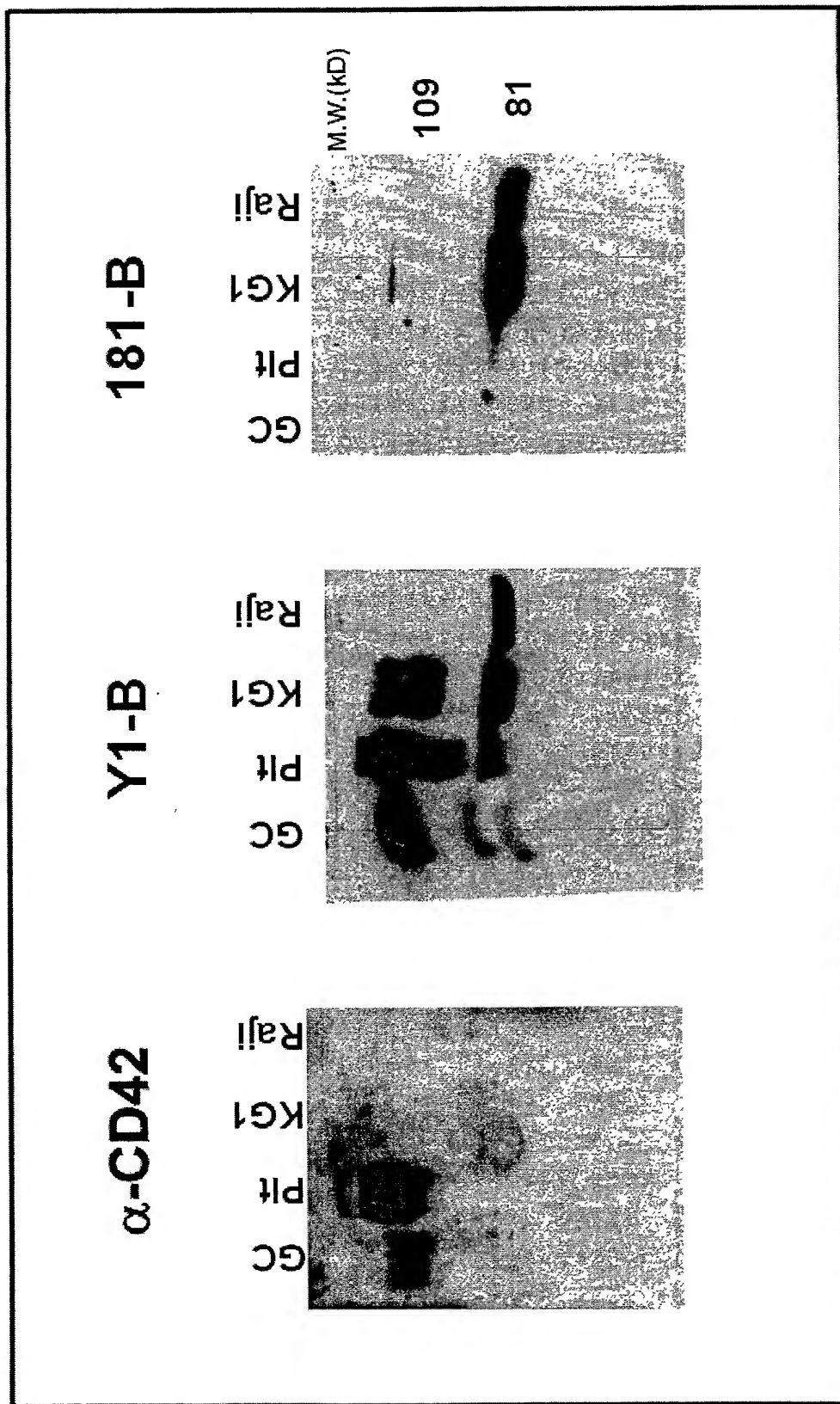
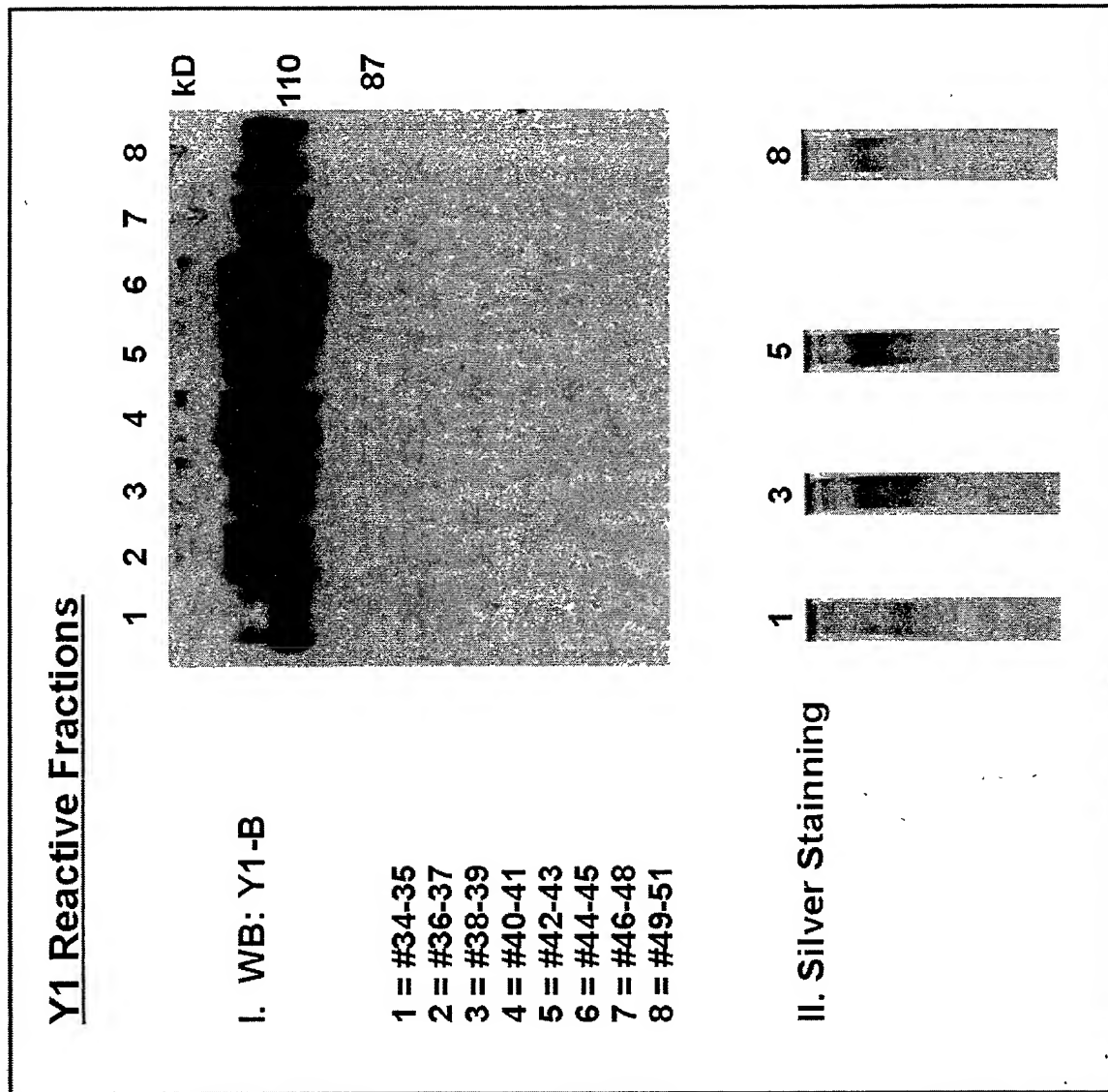


FIG. 21

**Y1-Ligand from KG1 membranes following
Immuno-Precipitation with Y1:
Purification on RP-HPLC**



Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

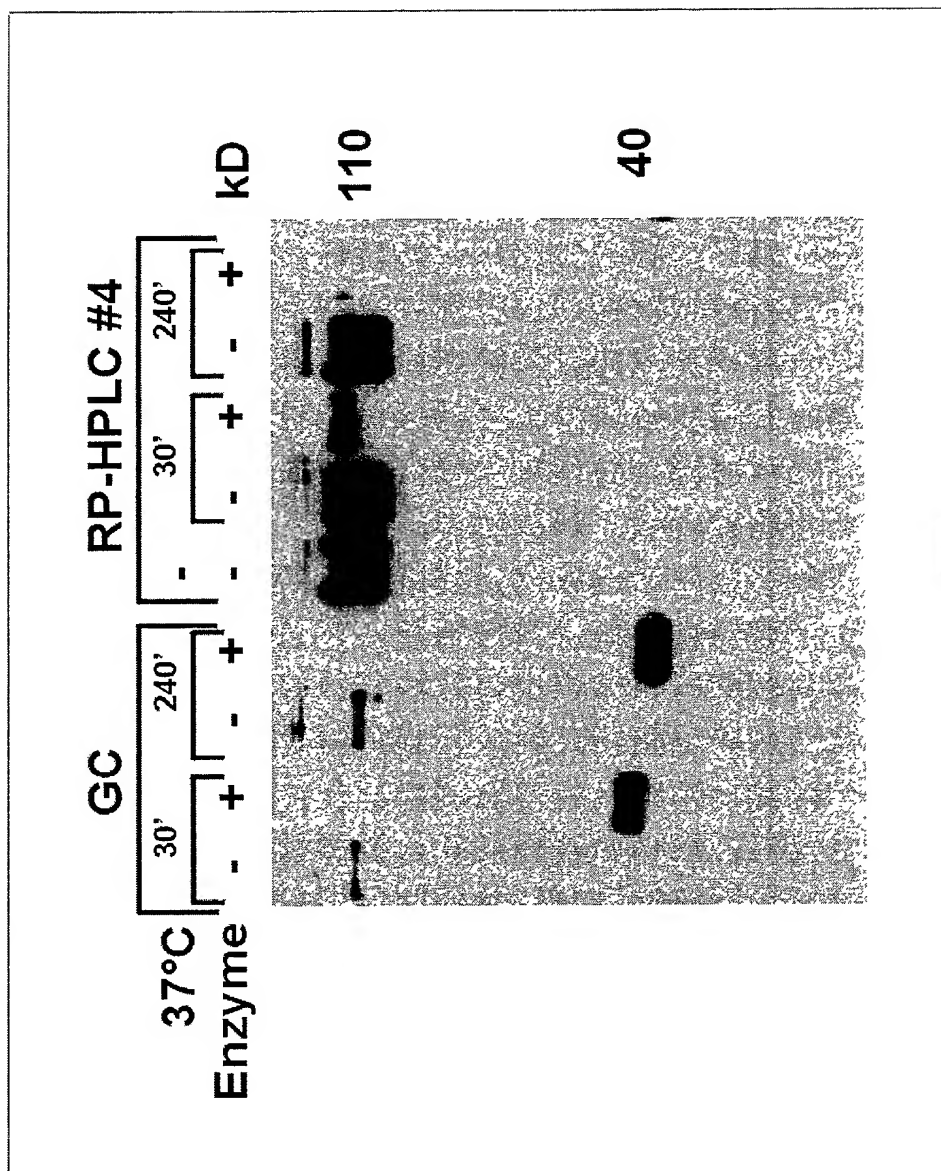
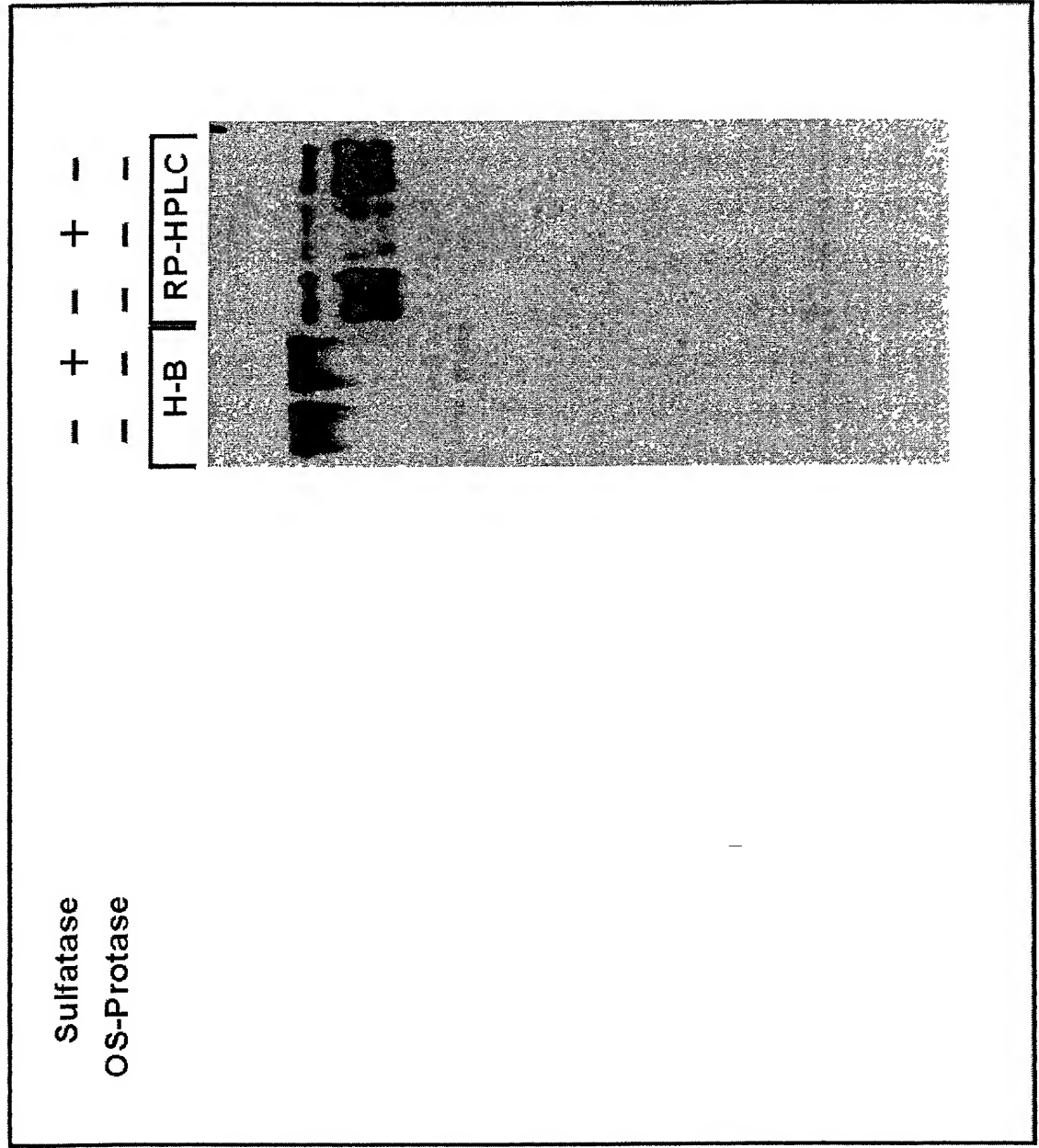


FIG. 22

FIG. 23

Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)



Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

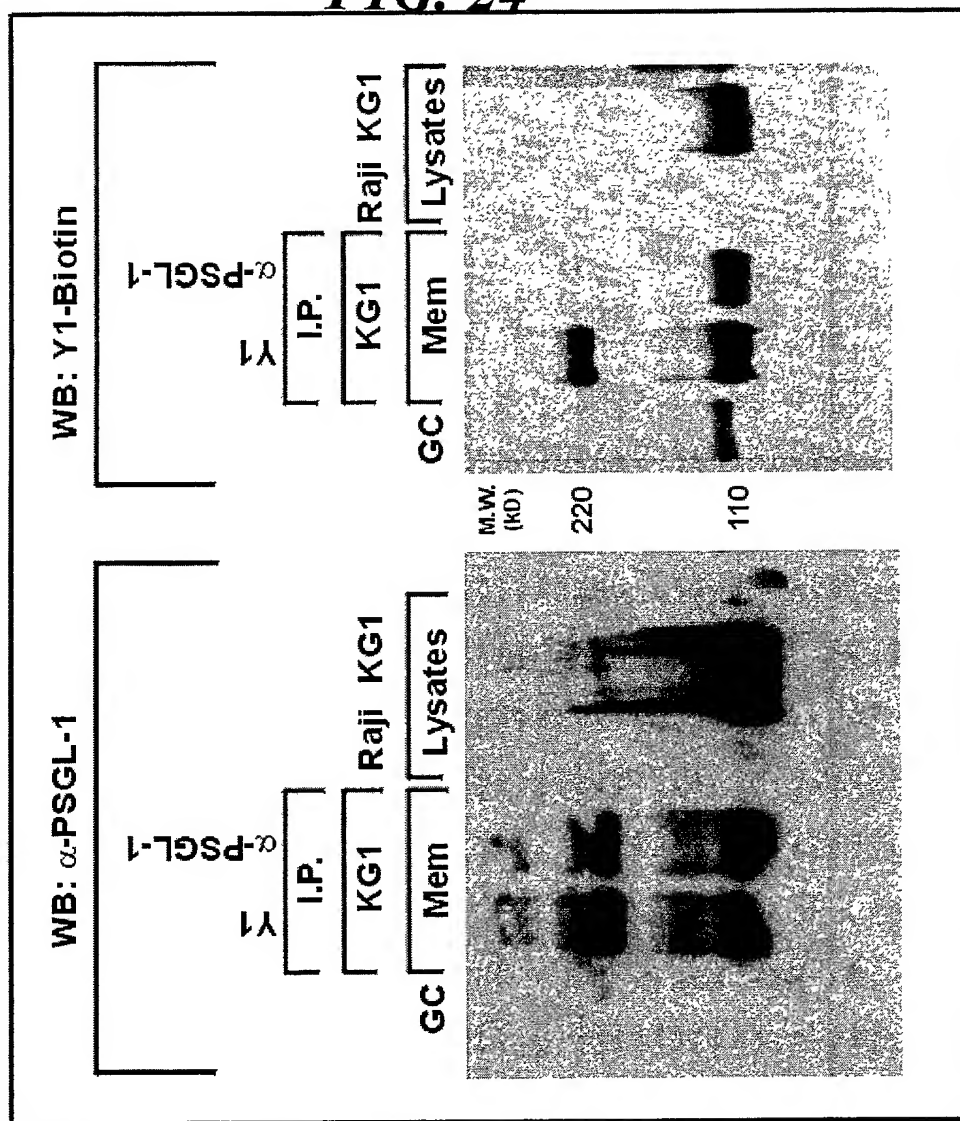
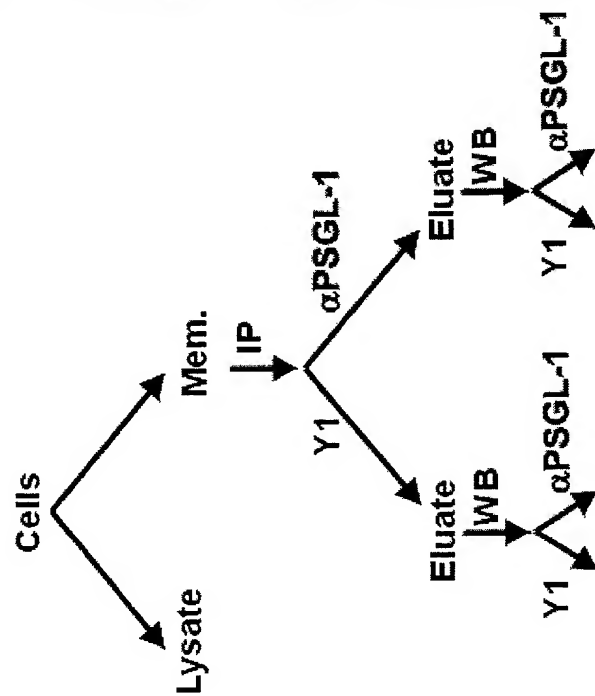
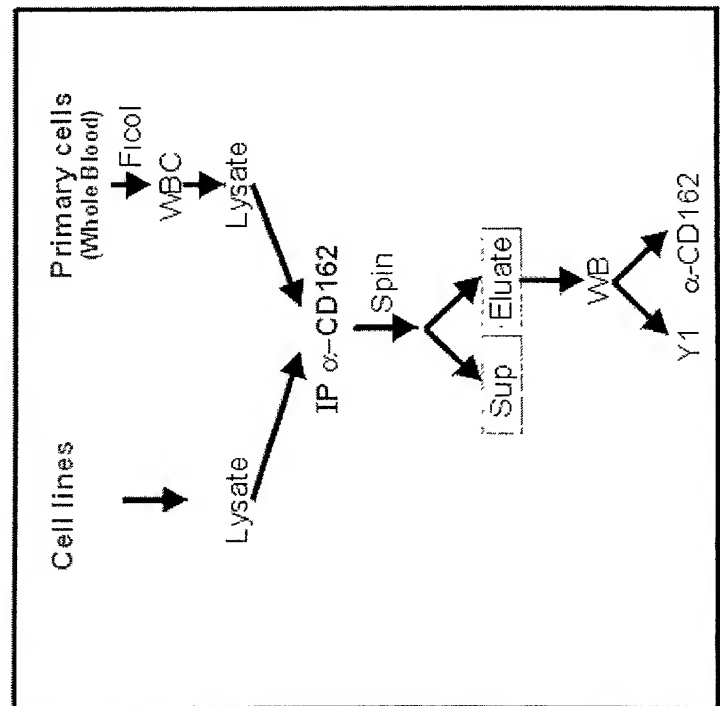


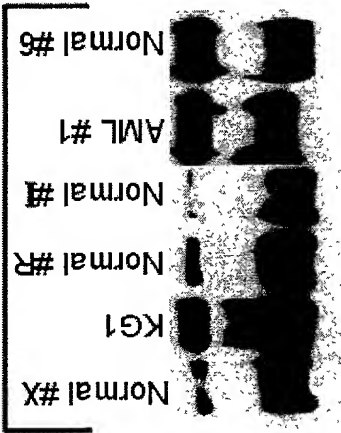
FIG. 25

α -CD162 and Y1:
Comparison between cells
from AML patient and normal
blood

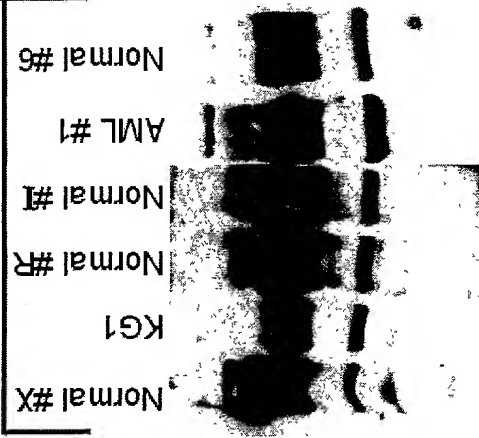


I

WB: α PSGL-1

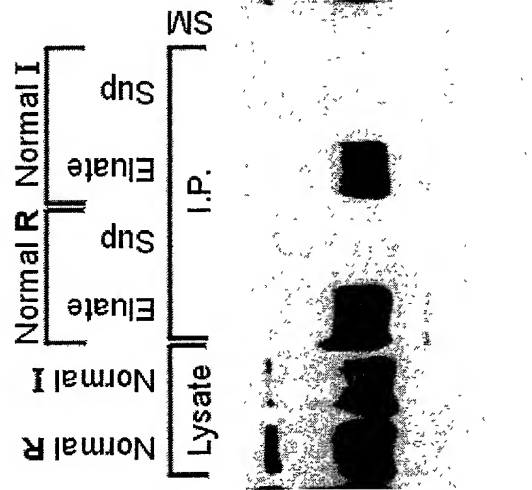


WB: Y1-B



II

WB: α PSGL-1



WB: Y1-B

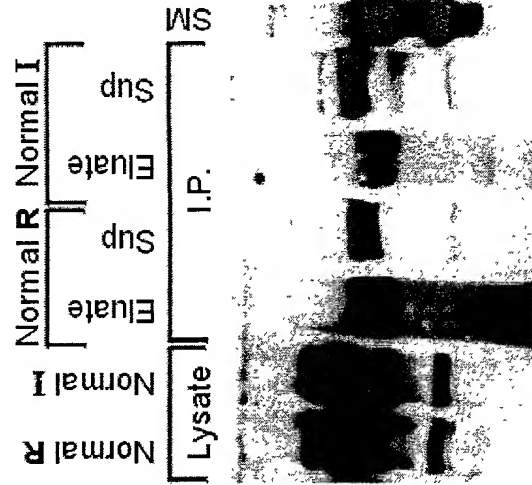
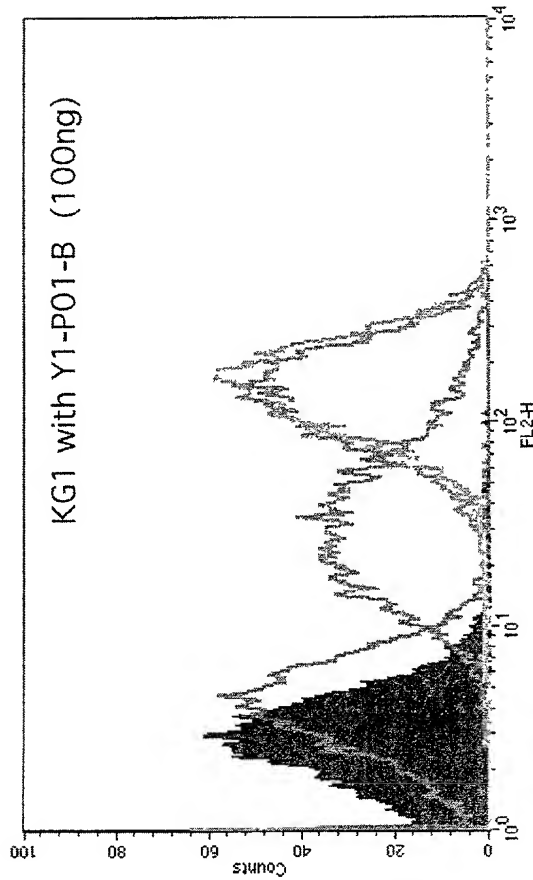
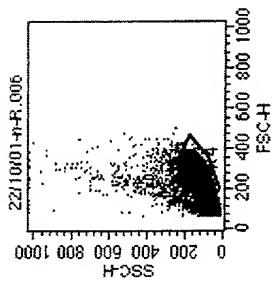
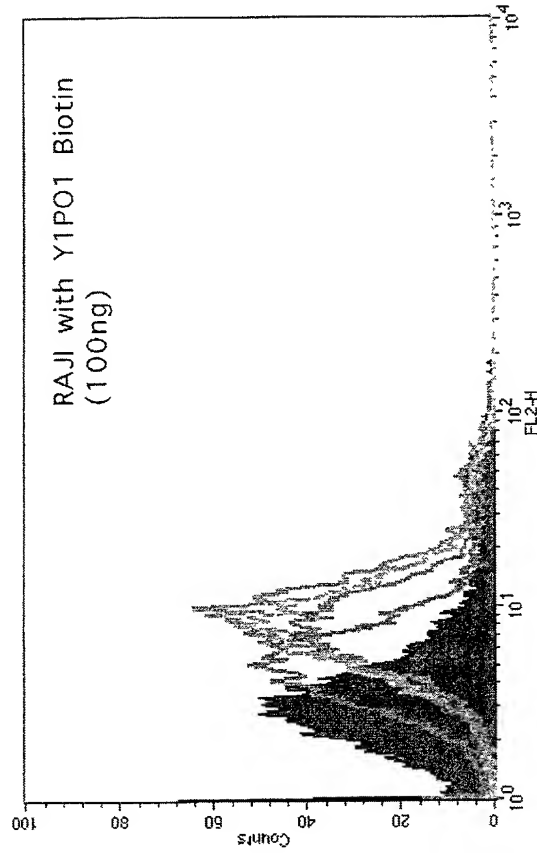
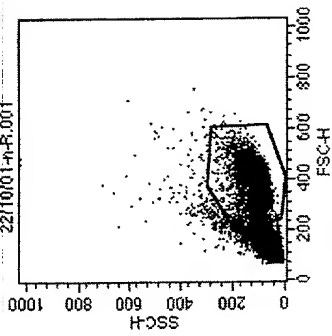


FIG. 26

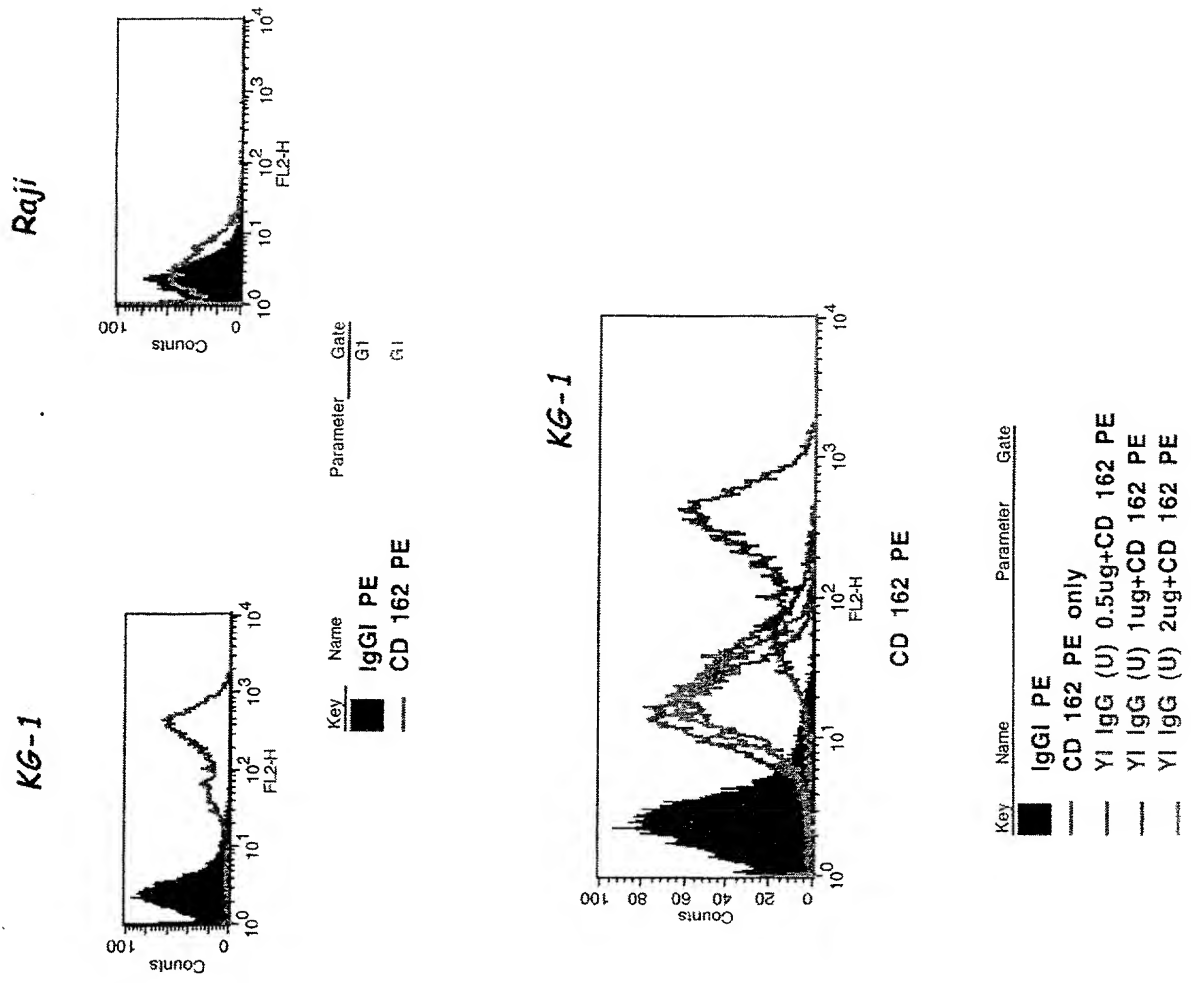


Key	Name	Parameter	Gate
	22/10/01-n-R.006	N01-B	
	22/10/01-n-R.007	P01-B	
	22/10/01-n-R.008	+KPL1	
	22/10/01-n-R.009	+PL1	
	22/10/01-n-R.010	+PL2	

Specificity of Y1 Binding: Analysis by FACS

- Binding of α PSGL1 (α CD162/KPL1); competition with Y1-IgG

FIG. 27



Specificity of Y1 Binding: Analysis by FACS

- Binding of
Y1-IgG;
competition
with α PSGL-1
(CD162 /KPL1)

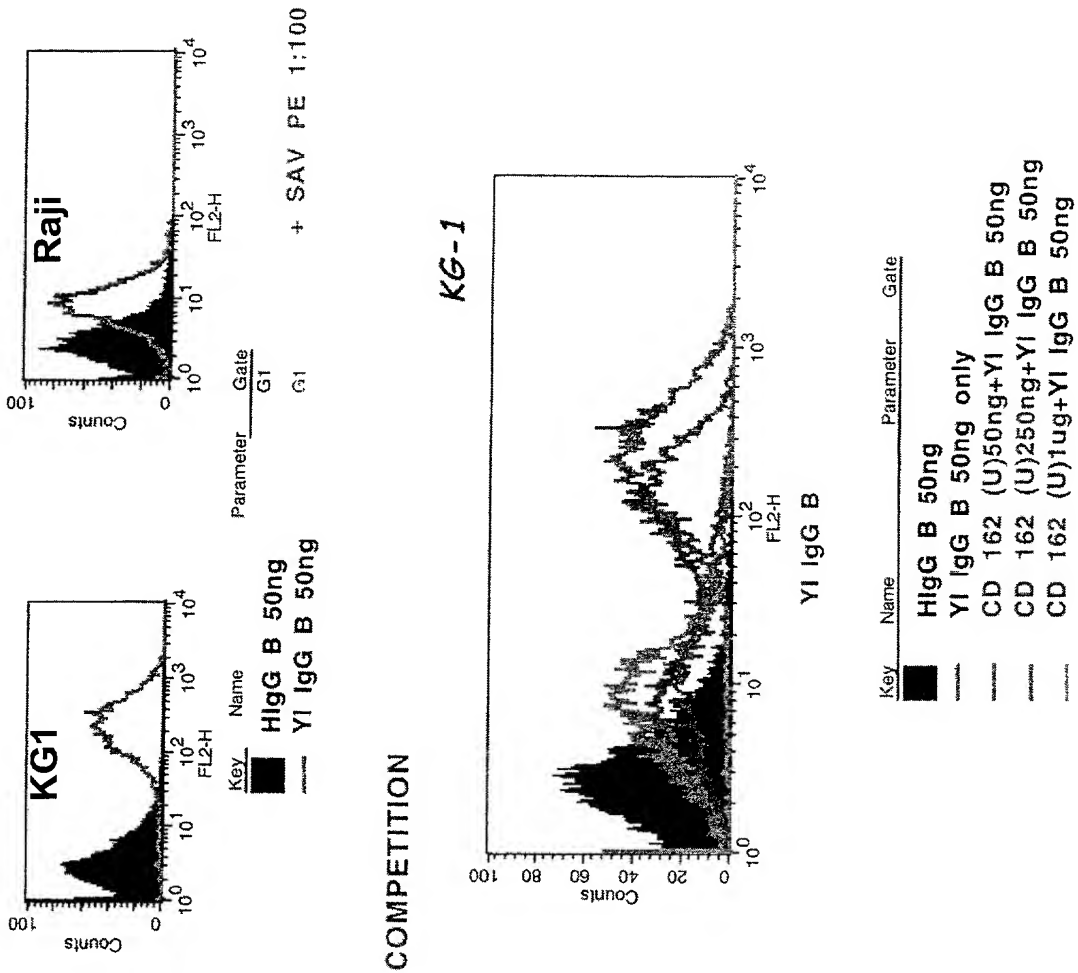


FIG. 28

FIG. 29

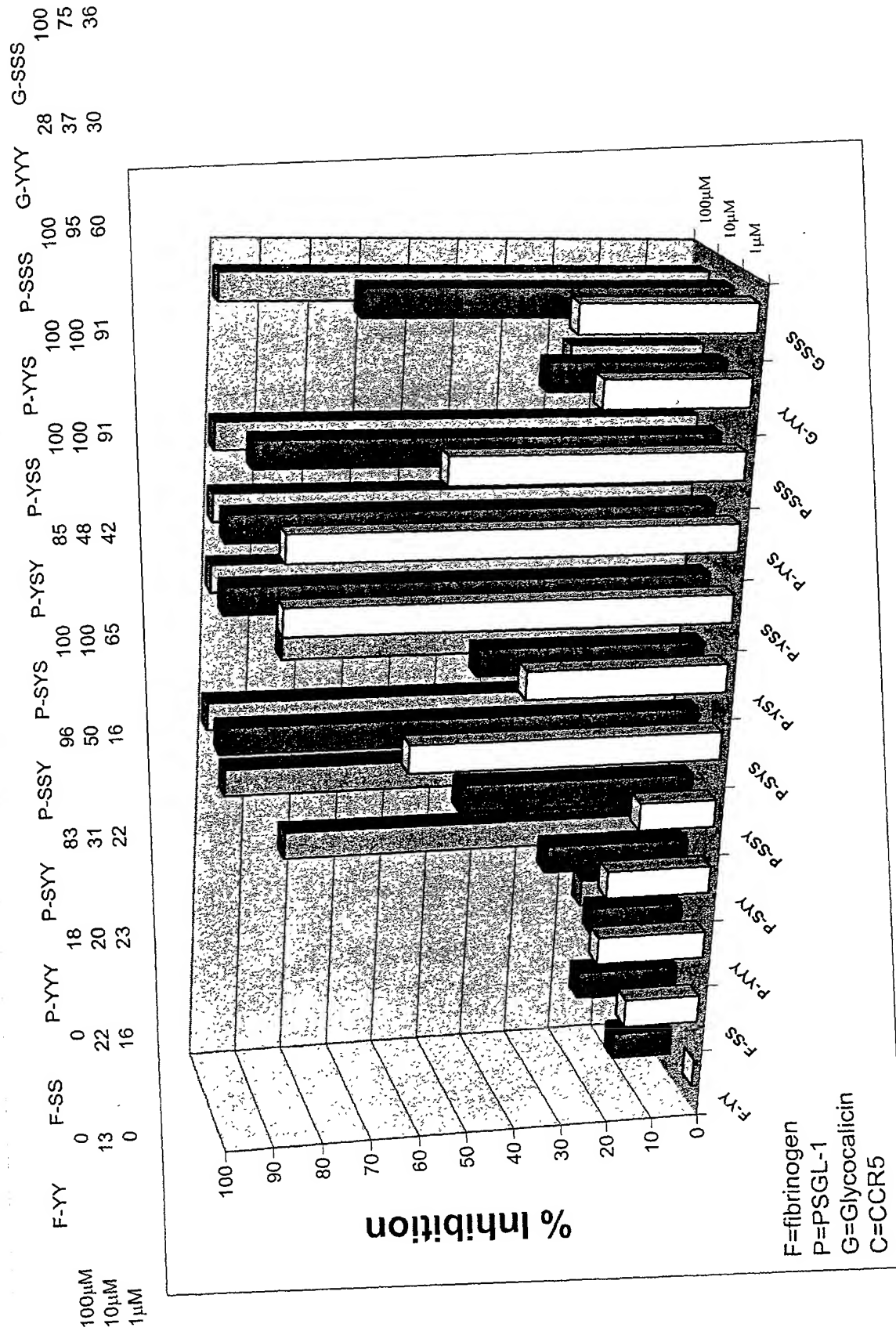


FIG. 30

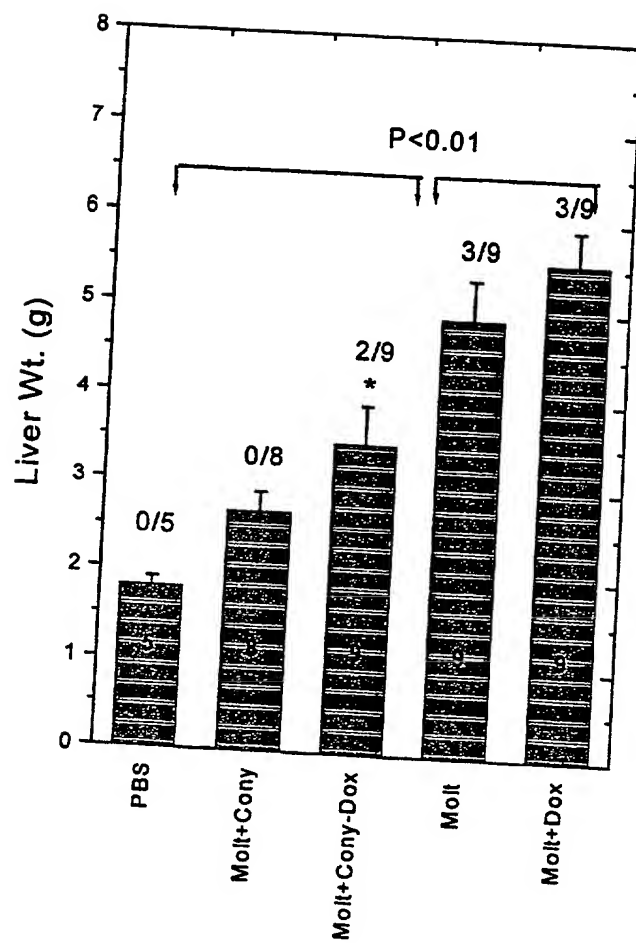


FIG. 31

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.

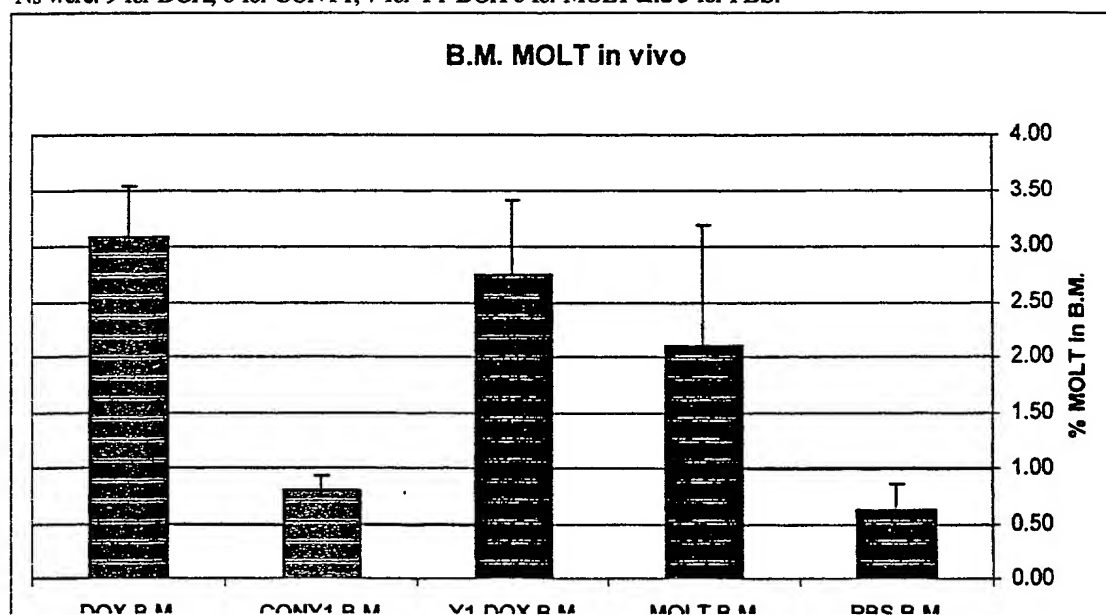
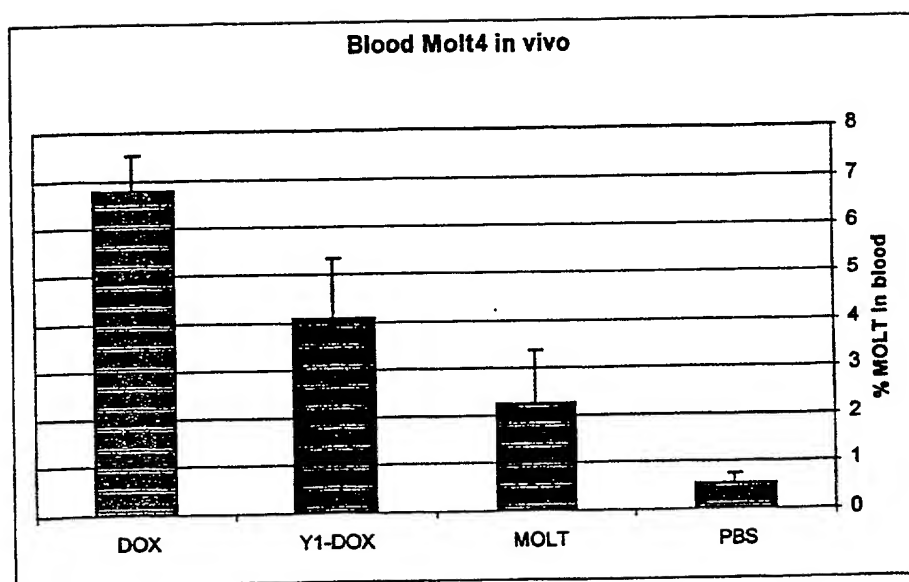


FIG. 32



****Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.**

FIG. 33

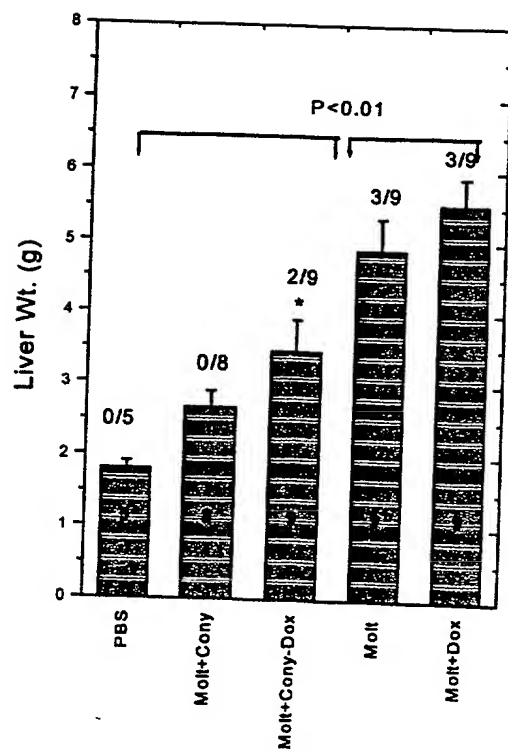


FIG. 34

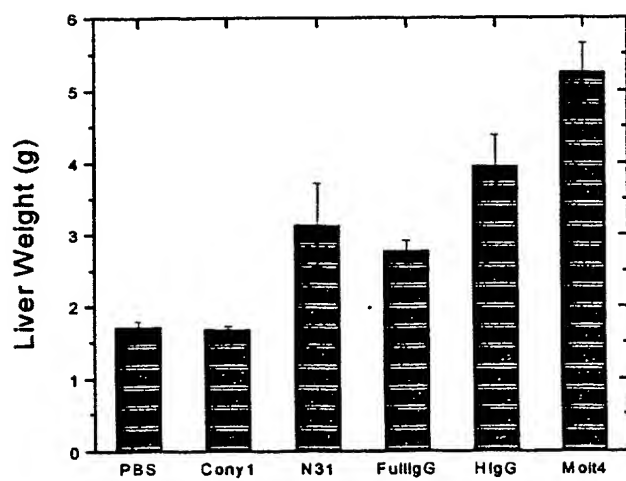


FIG. 35

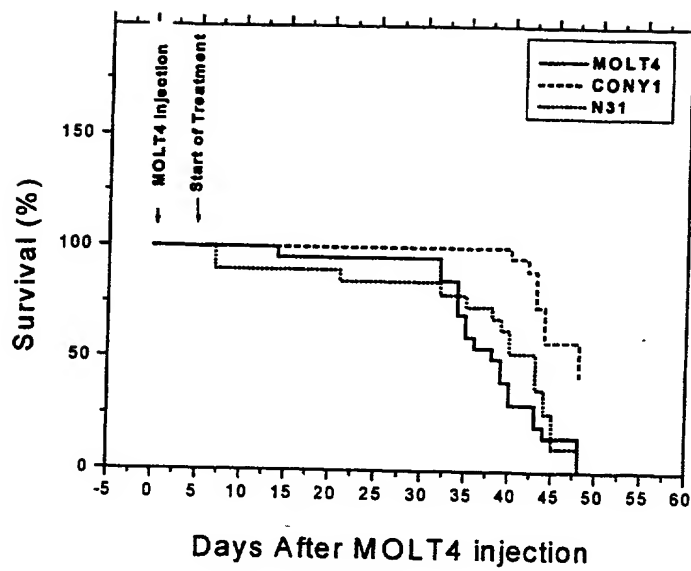


FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

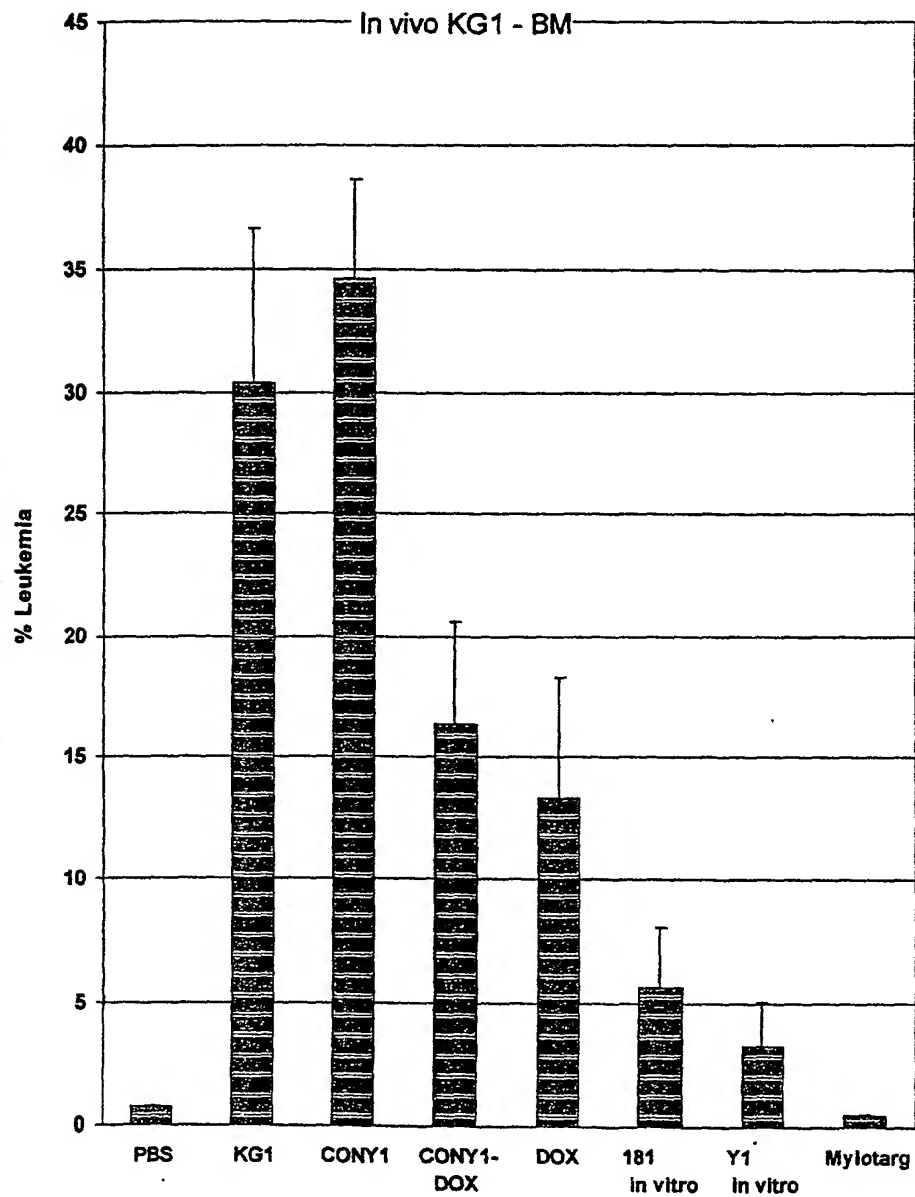
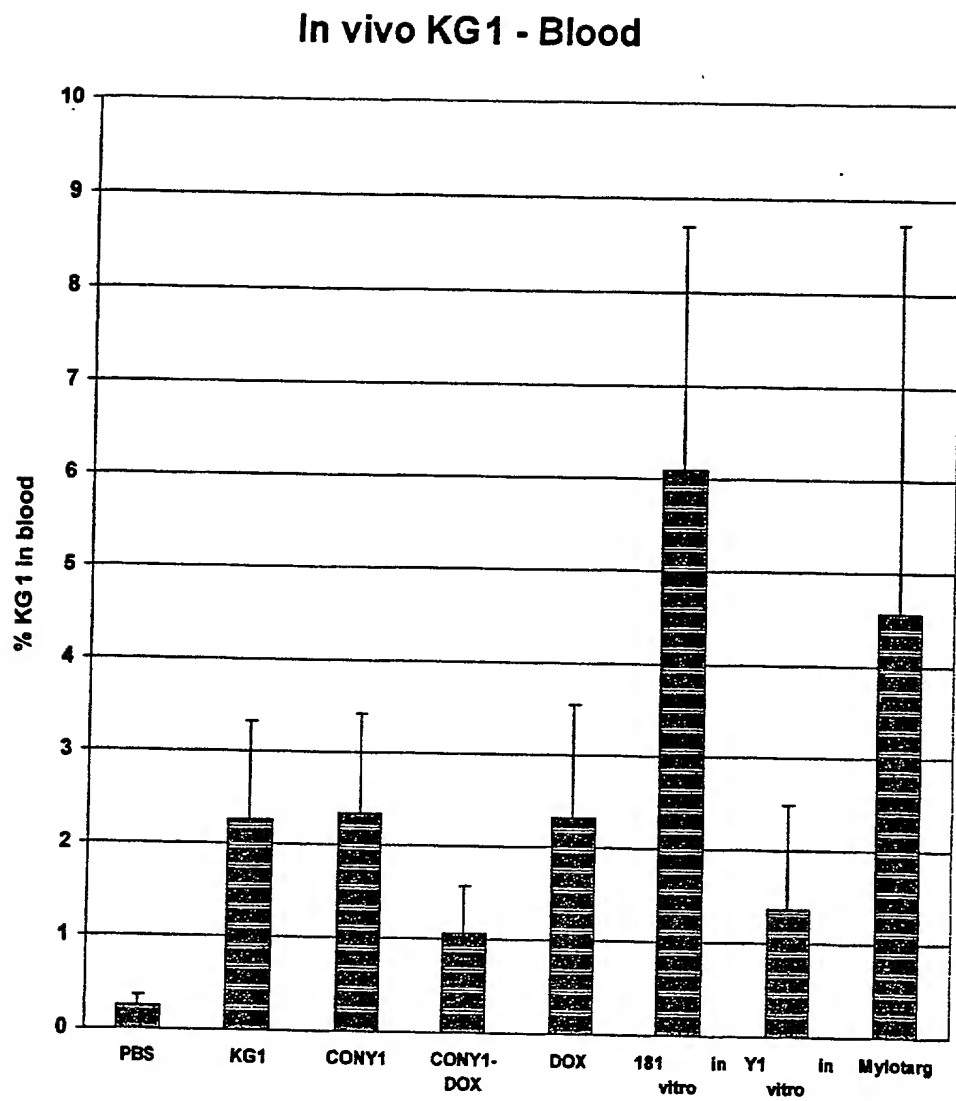


FIG. 37



****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

FIG. 38

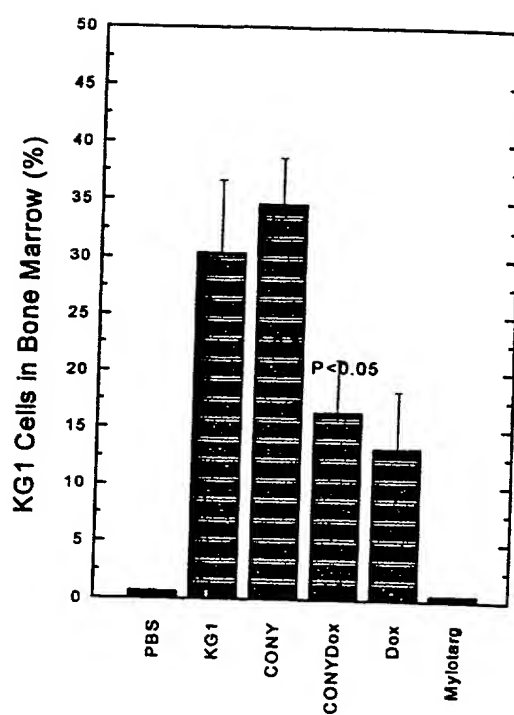
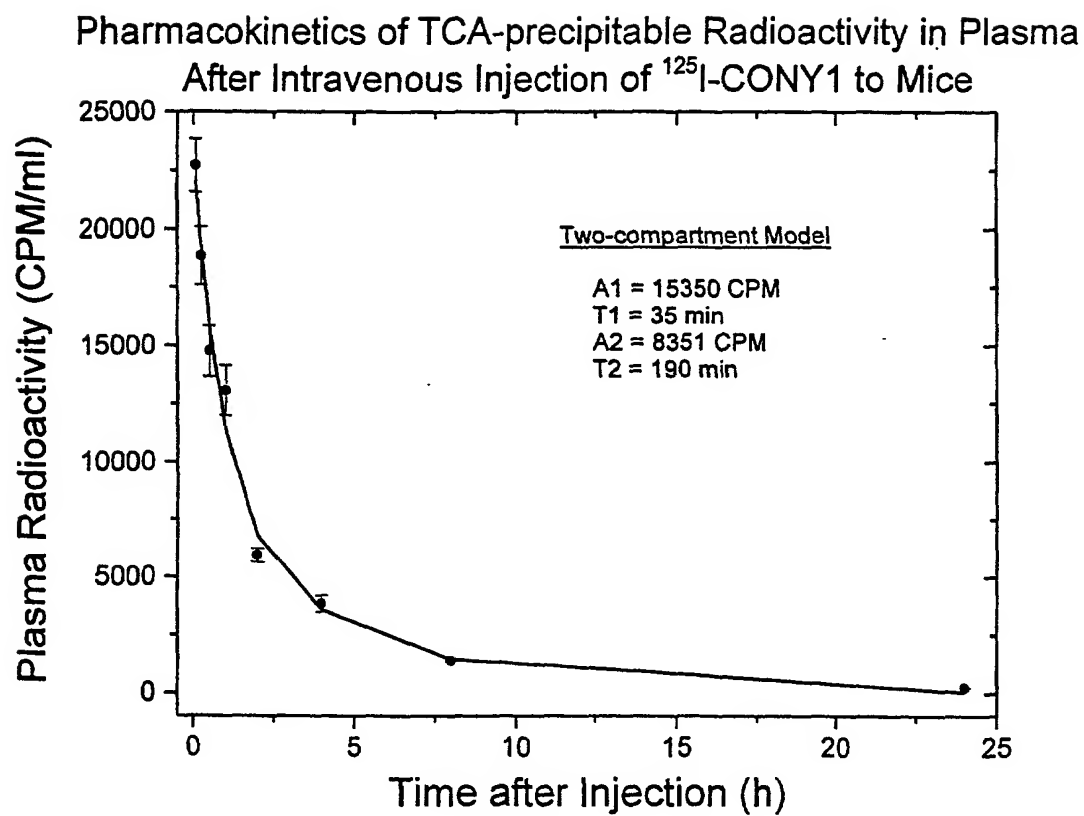


FIG. 39



[illegible]

FIG. 41

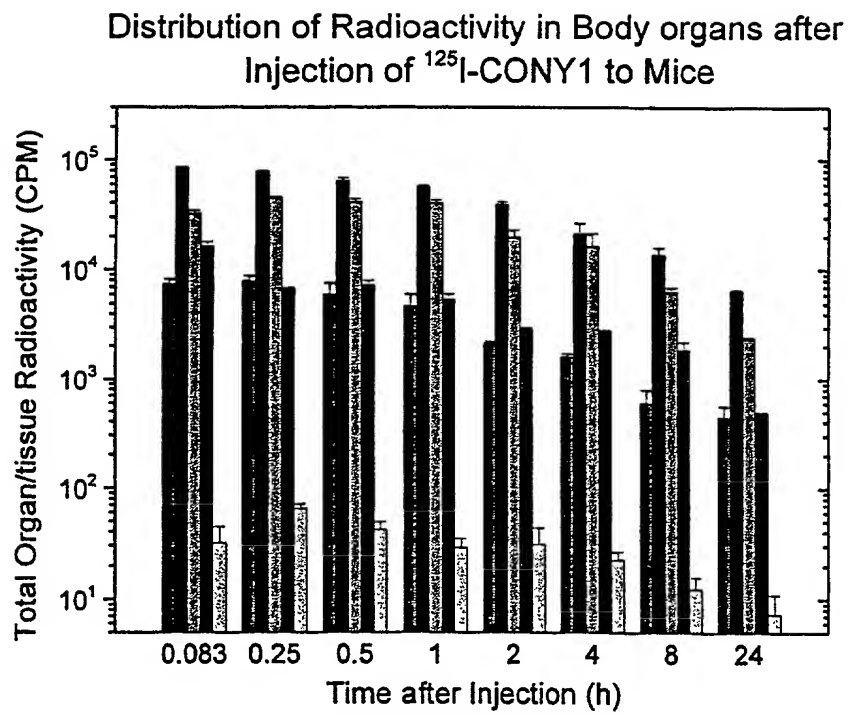


FIG. 42

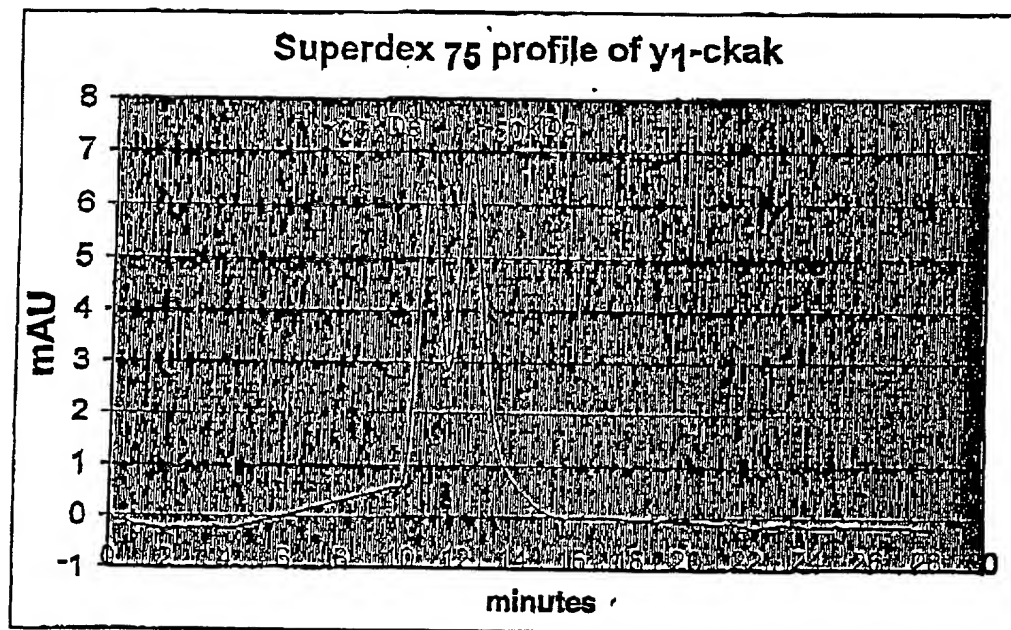
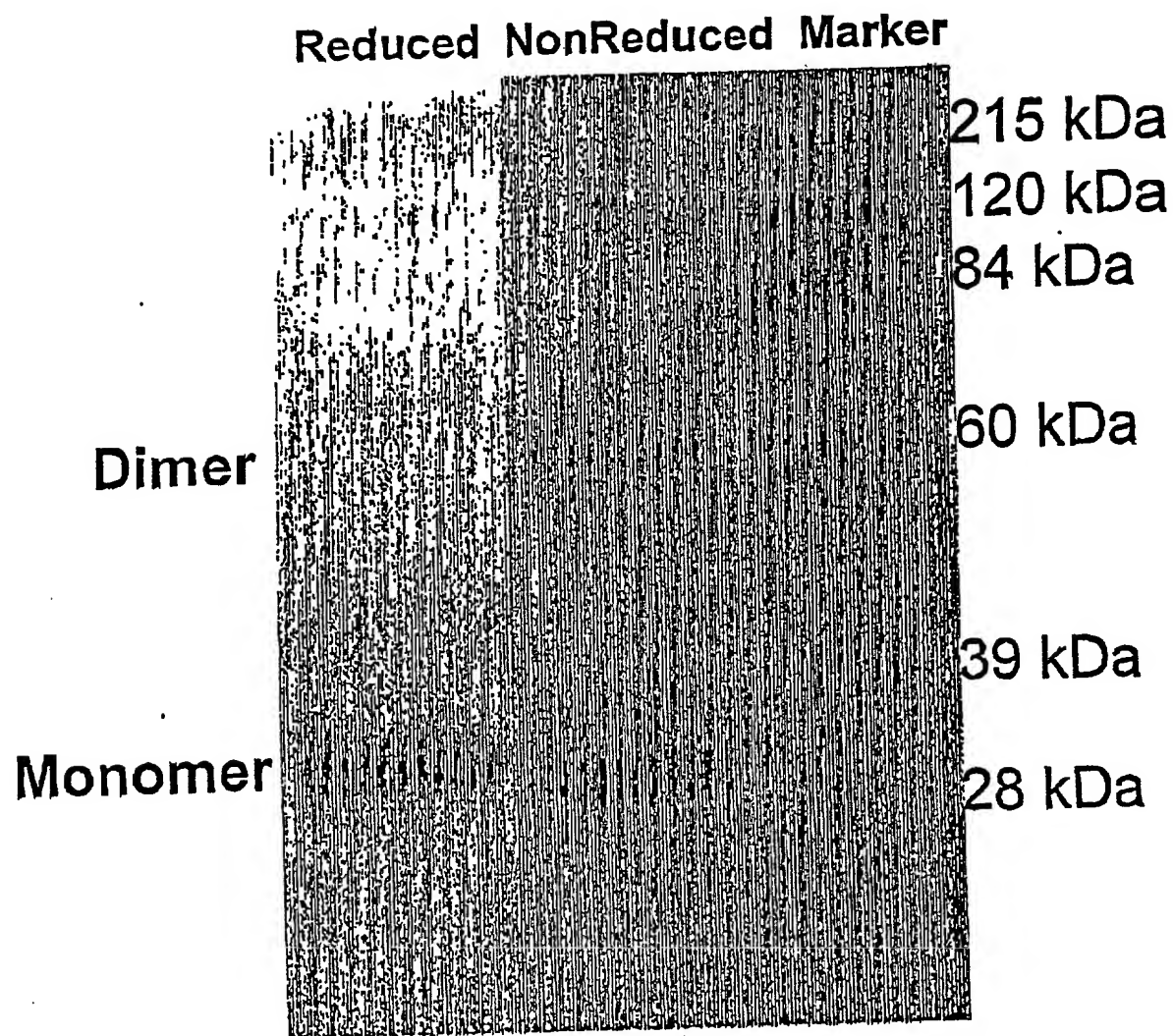


FIG. 43



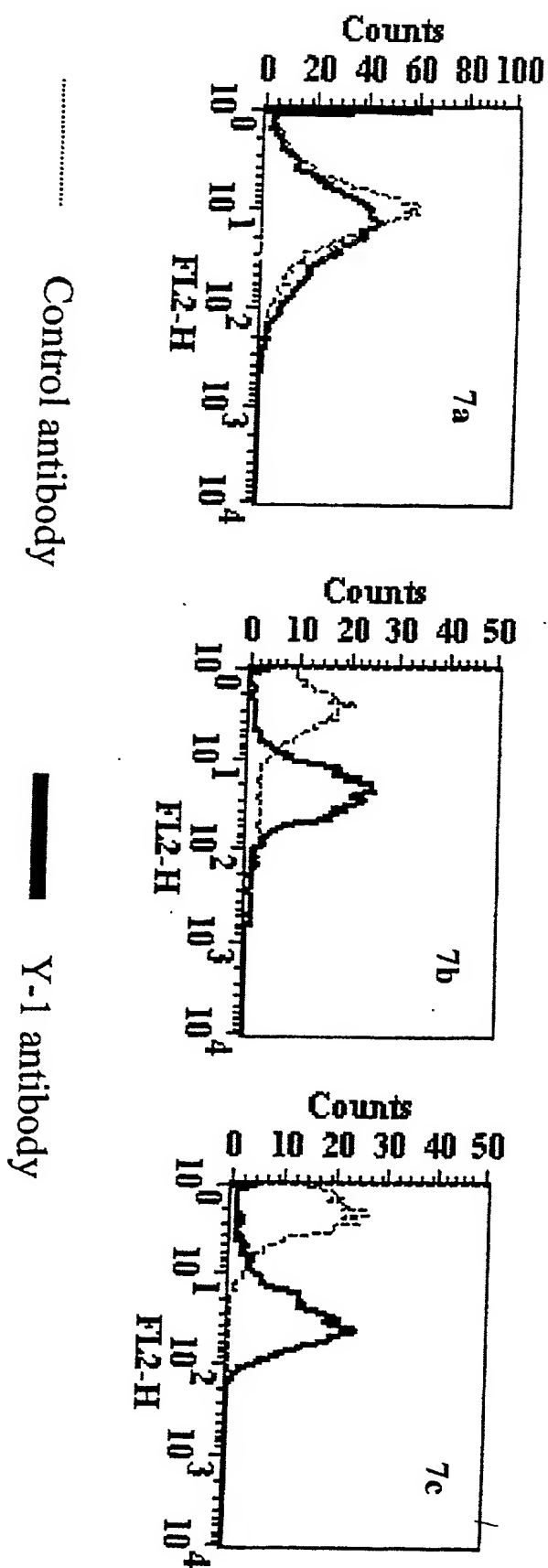
[illegible]

FIG. 45

Epitopes of anti-GPIb α antibodies

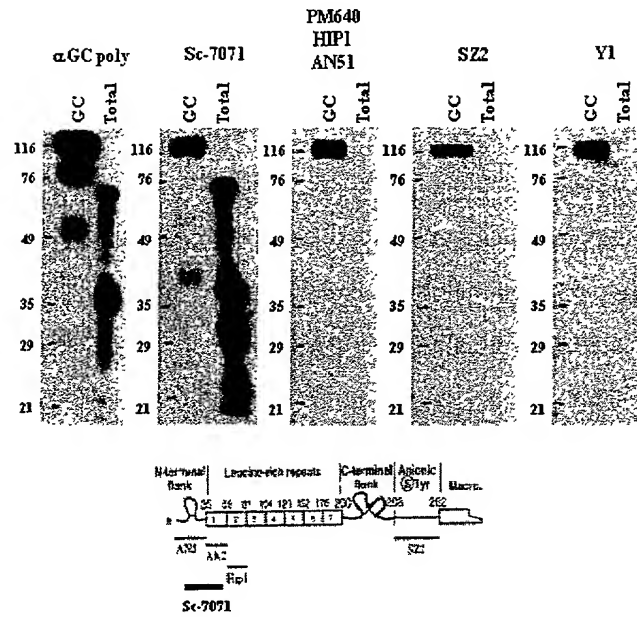


FIG. 46

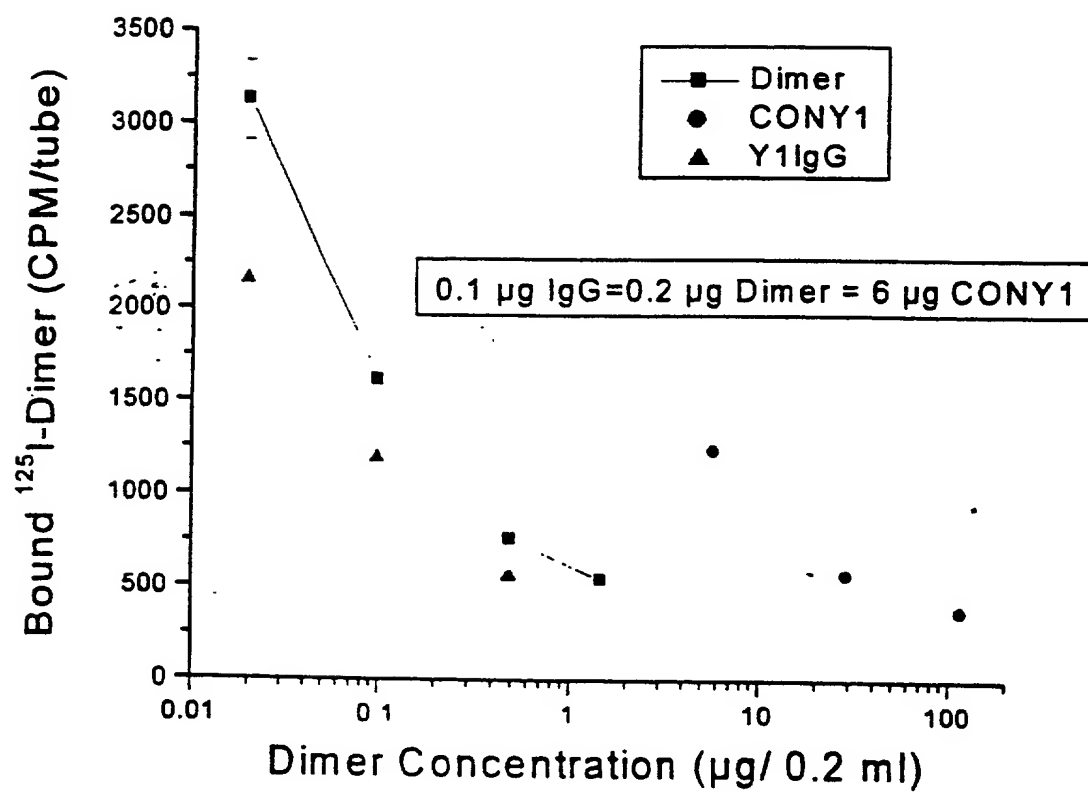
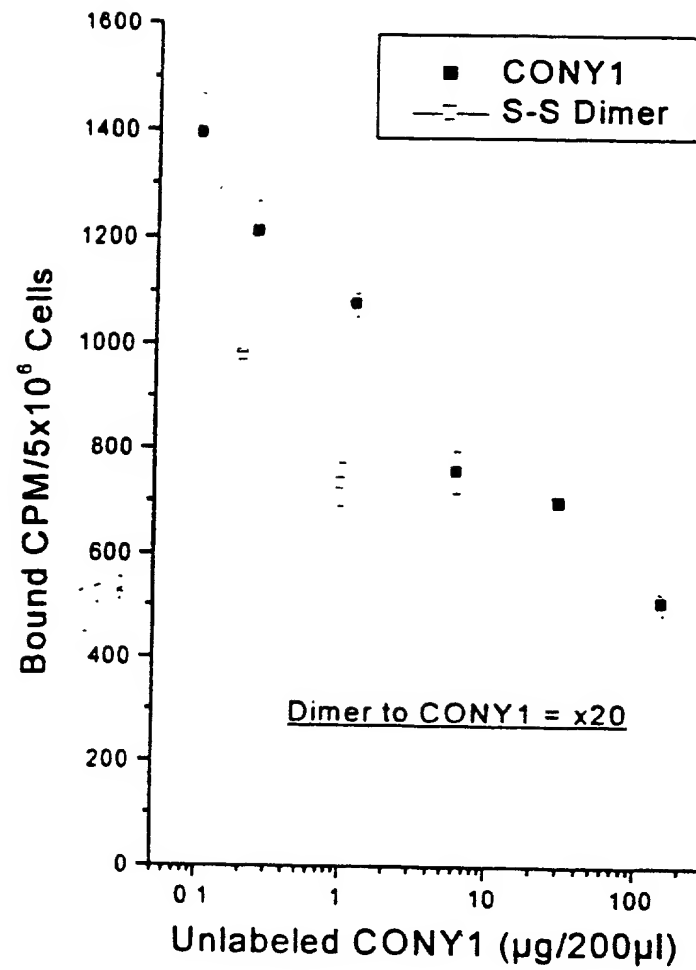


FIG. 47



[illegible]

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGGCTCTGCTGCTCCTOACCCCTCCTCACTCAGGACACAGGGTCTCTGGGCCGAT
1	M A W A L L L T L L T Q D T G S W A D
61	ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACTCAGGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAT
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACTGTCTAGTCTTTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGAGGAGATGACCAAGAACAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGAGAGAACAACCTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCTCTATAGCAAGCTCACCGTGCACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *

FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTCACCCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21	A E L T Q D P A V S V A L G Q T V R I T
1212	TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41	C Q G D S L R S Y Y A S W Y Q Q K P G Q
181	GCCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
161	A P V L V I Y G K N N R P S G I P D R F
241	TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81	S G S S S G N T A S L T I T G A Q A E D
301	GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101	E A D Y Y C N S R D S S G N H V V F G G
361	GGGACCAAGCTGACCGTCCTAGGTACGCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121	G T K L T V L G Q P K A A P S V T L F P
421	CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141	P S S E E L Q A N K A T L V C L I S D F
481	TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161	Y P G A V T V A W K A D S S P V K A G V
541	GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181	E T T T P S K Q S N N K Y A A S S Y L S
601	CTGACGCCTGAGCAGTGGGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201	L T P E Q W K S H K S Y S C Q V T H E G
661	AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221	S T V E K T V A P T E C S *

FIG. 49

	1	11	21	31	41	51	
1	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVYYCARVA	KTLMRQYSLW	GQGTLVTVSR	120
121	GGGSGGGGGS	GGGGSSELTQ	DPAVSVALGQ	TVRITCGDS	LRSYYASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNAA					

EVQLVESGGG

FIG. 50

		10	20	30	40	50	60
1		AtTaTTAcTc	gCGGCCcAGC	CgGCCcAGC	CGAGGTGCAG	CTGGTGGAGT	CTGGGGGAGG
3		L L L A A Q P A M A	E V Q L V E S G G G				
		70	80	90	100	110	120
1		CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA	GCCTCTGGAT	TCACCTTAG
3		L V Q P G G S L R L S C A A S G F T F S					
		130	140	150	160	170	180
1		CAGCTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG	GGGCTGGAGT	GGGTCTCAGC
3		S Y A M S W V R Q A P G K G L E W V S A					
		190	200	210	220	230	240
1		TATTAGGGT	AGTGGTGGTA	GCACATACTA	CGCAGACTCC	GTGAAGGGCC	GGTTCACCAT
3		I S G S G G S T Y Y A D S V K G R F T I					
		250	260	270	280	290	300
1		CTCCAGAGAC	AATTCCAAGA	ACACGCTGTA	TCTGCAAATG	AACAGCCTGA	GAGCCGAGGA
3		S R D N S K N T L Y L Q M N S L R A E D					
		310	320	330	340	350	360
1		CACGGCCGTG	TATTACTGTG	CAAGACCGG	GCAGAGTATT	AAGCGTATAT	GGGCGCAAGG
3		T A V Y Y C A R T G Q S I K R S W G Q G					
		370	380	390	400	410	420
1		TACCCGTGGT	ACCGTGTGCA	GAGGTGGAGG	CGGTTCAgGC	GGAgGTGgCT	CTGGCGGTGG
3		T L V T V S R G G G G S G G G G S G G G					
		430	440	450	460	470	480
1		CGGATCGTCT	GAgCTGACTC	AGGACCCTGC	TGTGTCTGTG	GcCTTGGGAC	AgACAGTCAG
3		G S S E L T Q D P A V S V A L G Q T V R					
		490	500	510	520	530	540
1		GATcACATGC	CAAGGAgACA	GCCTCAGAAG	CTATTATGCA	AGCTGGTACC	AGCAGAAGCC
3		I T C Q G D S L R S Y Y A S W Y Q Q K P					
		550	560	570	580	590	600
1		AGGACAGGCC	CCTGTACTTG	TCATCTATGG	TAAAAACAAC	CGGCCCTCAG	GGATCCCAGA
3		G Q A P V L V I Y G K N N R P S G I P D					
		610	620	630	640	650	660
1		CCGATTCTCT	GGCTCCAGCT	CAGGAAACAC	AGCTTCCTTG	ACCATCACTG	GGGCTCAGGC
3		R F S G S S S G N T A S L T I T G A Q A					
		670	680	690	700	710	720
1		GGAAGATGAG	GCTGACTATT	ACTGTAACTC	CCGGGACAGC	AGTGGTAACC	ATGTGGTATT
3		E D E A D Y Y C N S R D S S G N H V V F					
		730	740	750	760	770	780
1		CGGCGGAGGG	ACCAAGCTGA	CCGTCTAGG	TGCGGCCGCA	GAACAAAAAC	TCATCTCAGA
3		G G G T K L T V L G A A A E Q K L I S E					
		790	800	810	820	830	840
1		AGAgGAtCTG	AatGGGGCCG	CACgAACTG	TtGAATTTT	TAAGTTTAcC	T
3		E D L N G A A * N C * I F * V N					

Y16 SEQ ID NO: 210

FIG. 51

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL
81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LTVSRGGGG
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG
241 GGLNDIFEAQ KIEWHE

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FIG. 52

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK

FIG. 52 = GGGGGT